Acc 18/8/97

CUK-H03729.38-P024716

P-174

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corr 18/4/97

VOLUME 105 (10) — OCTOBER 1996

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ANNALS
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RADIOTHERAPY IN EARLY GLOTTIC CARCINOMA

BENIGN PAROXYSMAL POSITIONAL VERTIGO OF POSTERIOR SEMICIRCULAR CANAL: RESULTS IN 160 CASES TREATED WITH SEMONT'S MANEUVER

SEMON'S LAW REVISITED: AN ELECTROMYOGRAPHIC ANALYSIS OF LARYNGEAL SYNKINESIS

DIMENSIONS OF THE SINUS TYMPANI AND ITS SURGICAL ACCESS VIA A RETROFACIAL APPROACH

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June 1996

Ann Otol Rhinol Laryngol 105:1996

RADIOTHERAPY IN EARLY GLOTTIC CARCINOMA

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Radiotherapy is widely accepted as primary treatment in the T1 stage of glottic cancer, but controversy surrounds the proper approach to T2 lesions of the glottis. A retrospective review of 90 patients addresses treatment results for T1 and T2 lesions of glottic carcinoma managed by primary radiotherapy with 60 to 64 Gy from 1977 to 1989. Seventy-nine patients met the criteria for local control analysis with a minimum follow-up of 5 years. Radiotherapy alone controlled disease in 93% (43 of 46) of patients with T1 lesions and 18% (6 of 33) of those with T2 tumors (including 10 patients in whom radiotherapy was terminated at 40 Gy because of persistent tumor). Ultimate control of disease for T1 and T2 lesions, including surgical salvage, was 100% and 82%, respectively. Larynx preservation was achieved in 100% of T1 and in 45% of T2 lesions. Extension of tumor and impaired vocal cord mobility showed statistical significance for adverse prognosis ($p < .001$). This paper discusses how these results affect treatment of glottic carcinoma, particularly in the T2 stage.

KEY WORDS — carcinoma, glottis, radiotherapy.

INTRODUCTION

In about 75% of patients with laryngeal cancer, the tumor originates on the true vocal cord. Due to early interference with vocal fold motion, early stages are common at diagnosis of glottic cancer. According to the classification of the American Joint Committee on Cancer (AJCC),[1] "early cancer" of the glottis comprises Tis, T1, and T2 lesions without regional or distant metastasis.

For T1 tumors, radiotherapy is widely accepted as equally successful as compared with surgical techniques, but controversy exists on the proper approach to T2 lesions of the glottis.

This retrospective review of 90 patients will address treatment results for T1 and T2 lesions managed by primary radiotherapy and surgical salvage for failures. Local control, survival, patterns of recurrence, and efficacy of surgical salvage are analyzed. The prognostic significance of the various modalities of radiotherapy, vocal cord mobility, and the extent of disease is examined.

PATIENTS AND METHODS

Patients. At the Department of Radiotherapy of the University of Innsbruck, 97 previously untreated patients with glottic cancer received primary radiotherapy between February 1977 and December 1989. During this 13-year period, radiotherapy was almost the only form of treatment for early glottic cancer, and this population represents the vast majority (>95%) of all early glottic cancers diagnosed at the Department of Otolaryngology of the University of Innsbruck. Of the 97 cases reviewed, 5 cases of carcinoma in situ and 2 cases of verrucous carcinoma were primarily excluded to establish a homogeneous study group of 90 T1 and T2 lesions.

Patients were retrospectively staged according to the TNM system of the AJCC (1992)[1] by means of clinical records, radiologic studies, direct laryngoscopy, and biopsy performed at the parent institution: T1a was involvement of one true vocal cord (53 patients), T1b was involvement of both true vocal cords (2 patients), and T2 was extension to the supraglottis or subglottis (T2a if normal mobility [14 patients] and T2b if reduced mobility of the true vocal cord [21 patients]). Available data did not allow us to determine whether impaired vocal cord mobility was due to cricoarytenoid fixation or bulk muscle involvement of the paraglottic space. No regional or distant metastases were present.

The mean age at the beginning of radiotherapy was 63 years (range 25 to 84 years); all patients were white; 87 were male and 3 female.

The histologic diagnosis was invasive squamous cell carcinoma in 82 cases and microinvasive carcinoma in 8 cases.

Treatment. Until 1982, radiotherapy was administered with a cobalt 60 source, then with an 8-MeV linear accelerator. Irradiation was administered to bilateral ports with field sizes ranging from 5 × 5 to 6 × 6 cm. All patients had wedge compensation to maximize dose homogeneity. A plaster cast was used to immobilize patients during treatment. A single dose of 2.0 Gy in 46 patients or 2.5 Gy in 44 patients

---

From the Departments of Otolaryngology (Kanonier, Rainer, Thumfart) and Radiotherapy (Fritsch), University of Innsbruck, Innsbruck, Austria.

Presented at the meeting of the American Laryngological Association, Palm Desert, California, April 29-30, 1995.

TABLE 1. SALVAGE PROCEDURES AND SUCCESS RATE IN EARLY GLOTTIC CARCINOMA

Stage	*No. of Radiation Failures*	*Tumor Persistence at 40 Gy*	*Salvage Procedures**	*Failure of Salvage*
46 T1	3	0	1 Cordectomy	0
			1 Hemilaryngectomy	0
			1 Additional radiation	0
12 T2a	5	2	1 Cordectomy	0
			1 Hemilaryngectomy	0
			4 Total laryngectomy	1
21 T2b	12	8	3 Cordectomy	0
			1 Hemilaryngectomy	0
			13 Total laryngectomy	2
			1 Total laryngectomy combined with radical neck dissection	0

*One T2a patient and 2 T2b patients died without attempt at salvage operation because of distant metastatic disease, unresectable primary, and presence of secondary malignant melanoma, respectively.

was administered to both lateral fields daily for 5 days a week. This variation of the daily dose represents the change in attitude concerning the optimal fraction during the study period. The average duration of radiotherapy was 6 weeks; in 16 patients treatment was extended beyond 6 weeks because of local side effects. The total tumor dose ranged between 60 and 64 Gy, but in 10 patients radiotherapy was cancelled and classified as ineffective because tumor persistence was diagnosed after administration of 40 Gy. Restaging of the larynx by microlaryngoscopy and biopsy was routinely performed under general anesthesia after application of 40 Gy. These 10 patients with tumor persistence subsequently underwent surgery (Table 1). After completion of treatment, the patients were routinely controlled at regular intervals to rule out a locoregional recurrence, distant metastasis, or secondary neoplasm.

Methods of Analysis. Patients who died of a different illness within 5 years of treatment without evidence of disease were excluded from local control analysis. To complete a minimum follow-up of 5 years, all patients were asked by letter to return for examination or to name their physician, who was contacted by one of the authors for further information. If death had occurred, further information was provided by the medical records. Six patients died of a different illness after less than 5 years, and 5 patients disappeared from follow-up (7 with T1 disease and 4 with T2). Thus, a total of 79 patients was evaluated with a minimum follow-up of 5 years.

The 5-year corrected actuarial survival and locoregional control at 5 years with and without salvage were assessed in this population. Statistical significance was determined by the two-sided Fisher's exact test.

RESULTS

Seventy-nine patients with T1 (46) or T2 (33) squamous carcinoma of the laryngeal glottis treated primarily with radiotherapy for cure were available for analysis. The 5-year corrected actuarial survival was 100% in the T1 group and 82% in the T2 group. For survivors, the mean follow-up period was 7 years (range 5 to 15 years). Full-course radiotherapy controlled disease in 93% (43 of 46) of patients with T1 lesions and 26% (6 of 23) of those with T2 tumors. For patients failing radiotherapy, the median intervals to tumor recurrence following completion of irradiation were 16 months in T1 disease (range 15 to 57 months) and 13 months in T2 (range 4 to 36 months). Tumor persistence after application of 40 Gy occurred only in T2 carcinomas and was evident in 2 of 12 (17%) T2a lesions and 8 of 21 (38%) T2b lesions. The different forms of salvage procedures are shown in Table 1.

Three failures were observed in T1 lesions. All occurred in the glottis and were treated successfully by cordectomy, hemilaryngectomy, and additional irradiation with 18 Gy, respectively. In the third patient the application of 30 Gy was planned, but radiotherapy was terminated at 18 Gy because of urosepsis. Salvage therapy improved primary control in the T1 stage from 93% to 100%. Laryngeal conservation was 100%.

There were 17 failures in 23 T2 lesions with completed irradiation (5 of 10 T2a tumors and 12 of 13 T2b tumors, including 1 recurrence in the cervical lymph nodes in each group). Persistent disease at 40 Gy was noted in 10 patients; it is of interest that all 10 of these patients remained free of disease after surgical intervention. Salvage surgery in T2 carcinomas consisted of cordectomy in 4 cases, hemilaryngectomy in 2 cases, and total laryngectomy in 18 cases, in 1 case combined with radical neck dissection. Three patients died without an attempt at a salvage procedure because of distant metastatic disease, an un-

TABLE 2. PROGNOSTIC SIGNIFICANCE IN EARLY GLOTTIC CARCINOMA

Variables		*Total No.*	*Local Failure + Persistence*	*p*
T stage	T1	46	3 (7%)	<.0001
	T2	33	27 (74%)	
No. of subsites	≤3	66	19 (29%)	.0002
	>3	13	11 (85%)	
Involvement of anterior commissure	Yes	15	13 (87%)	<.0001
	No	64	17 (27%)	
Subglottic extension	Yes	5	3 (60%)	.28 (NS)
	No	74	27 (36%)	
Vocal cord mobility	Normal	58	10 (17%)	<.0001
	Reduced	21	20 (95%)	
Fraction	2.0 Gy	41	18 (44%)	.26 (NS)
	2.5 Gy	38	12 (32%)	
Field size	5 × 5 cm	68	28 (41%)	.15 (NS)
	6 × 6 cm	11	2 (18%)	
Tumor dose (10 patients with tumor persistence at 40 Gy excluded)	60 Gy	62	18 (29%)	.78 (NS)
	64 Gy	7	2 (29%)	
Duration of radiotherapy (tumor persistence at 40 Gy excluded)	5 + 6 wk	53	14 (26%)	.36 (NS)
	>6 wk	16	6 (37%)	

Two-sided Fisher's exact test.
NS — not significant.

resectable primary, and presence of a secondary malignoma (malignant melanoma), respectively. Total laryngectomy did not control disease in 3 cases. Overall, surgical salvage improved the ultimate control rate in 21 of 24 T2 lesions (87.5%) with failure of irradiation.

For evaluation of prognostic factors the following parameters were analyzed: tumor extension, involvement of the anterior commissure, subglottic extension, vocal cord mobility, histology, tumor dose, field size, and duration of radiotherapy. Tumor extension, measured by the number of subsites (anterior, middle, and posterior parts of glottic, supraglottic, and subglottic regions), involvement of the anterior commissure, and vocal cord mobility were significant prognostic factors of local control. No prognostic significance was observed for subglottic extension or modalities of radiotherapy (Table 2).

TABLE 3. INCIDENCE OF SECOND PRIMARIES IN EARLY GLOTTIC CARCINOMA

Time to Second Primary Tumor	*Lung*	*Esophagus*	*Head and Neck**	*Stomach*	*Malignant Melanoma*
0-2 y			2		
2-5 y					1
>5 y	2	1	3	1	
Total	2	1	5	1	1

*Recurrence outside irradiation field.

Larynx preservation was achieved in 100% of T1 tumors. With regard to 18 total laryngectomies (55%) in 33 T2 glottic cancers, lung-powered glottic voice preservation was achieved in 8 of 12 T2a tumors and in 7 of 21 T2b tumors. Since objective measurement of voice quality 5 years after definitive radiotherapy was possible in only a few patients, these data were omitted from analysis.

A second malignancy (Table 3) occurred in 10 patients after a median interval of 6 years (range 6 months to 13 years), leading to death in 7 patients (1 patient with evidence of tumor at the site of the primary in the larynx died of malignant melanoma); 90% of these second primary tumors were located in the aerodigestive tract.

DISCUSSION

There is still an ongoing debate about the optimal treatment of early glottic carcinoma. In the case of T1 lesions of glottic cancer, radiotherapy and surgical excision produce equal results, with local control rates above 90% in both treatment modalities. Radiotherapy of T1 lesions in our series effected a local control rate of 93%, with successful salvage in 100%.

A significant difference was observed in our results for T2 lesions of glottic cancer. In the 23 patients receiving a therapeutic dose of 60 to 64 Gy, 17 recurrences occurred, producing a failure rate of 74%. The failure rate was especially high for T2b tumors, where 12 of 13 tumors recurred (92%). With regard to more recent reports of control rates of T2 lesions between 50% and 75% with total tumor doses of 66 to 76 Gy,[2-5] the dose of 60 Gy used in 90% of the present series now seems inappropriate for T2 lesions, but during the treatment period (1977 to 1989) a dose of 60 Gy was the standard regimen.

The concept that tumor persistence after application of 40 Gy is considered a poor response to radiotherapy has now generally been abandoned. This strategy of altering the therapy course was based on observations by Teshima et al[6] and Fletcher et al,[7] who reported that persistent evidence of tumor at 40 Gy is associated with significant probability of a recurrence after completion of irradiation. In these 10 patients (30% of T2 tumors), subsequent surgical therapy achieved a control rate of 100%. It cannot be ruled out that a substantial portion of those patients classified as nonresponders because of tumor persistence at 40 Gy would also have been free of disease after completion of radiotherapy.

Tumor volume, vocal cord mobility, subglottic extension, field size, and tumor dose are claimed as prognostic factors by other authors.[2-4,8-14] In our series, the prognostic factors were tumor stage, number of subsites, involvement of the anterior commissure, and impaired vocal cord mobility. These findings are supported by numerous authors, who place great emphasis on vocal cord mobility.[3-5,10,12,13,15-19] Kersh et al[20] report local control in 44%, and Krawczyk et al[21] in 39%, of T2b tumors undergoing radiotherapy. Steiner[22] describes a trend to include tumors with reduced vocal cord mobility in the T3 category, because their prognosis is closer to that of these tumors. This observation also holds true for our population of T2b carcinomas.

In contrast to the results of radiotherapy alone, the 5-year cure rate with surgical salvage was 100% in T1 lesions and 82% in T2 lesions. The number of 18 total laryngectomies (55%) in 33 T2 tumors, however, is disappointing with regard to the loss of lung-powered vocal function. We suppose that in a substantial number of patients the larynx would have been saved if conservation surgery had been performed as a first treatment option. The importance of obtaining information about resection margins in surgical procedures should not be underestimated, since revision surgery remains practicable in the case of positive margins, with total laryngectomy reserved for therapy-resistant cases.[22-24]

Recent reports of the senior author[23,24] concerning the efficacy of endoscopic laser surgery of early glottic carcinoma have demonstrated excellent results in T1 and T2 glottic cancer, with cure rates of 95% in T1 and 85% in T2 cancers after 2 years. These results are supported by numerous other authors.[22,25-30] On the basis of these figures, a surgical approach using transoral laser resection as primary treatment for T1 and also T2 glottic cancer (T2a and T2b lesions) seems promising for the preservation of more functional larynges as compared to radiotherapy.

CONCLUSION

This review of treatment results of primary radiotherapy with 60 to 64 Gy in early glottic cancer revealed a satisfactory control of T1 glottic lesions, but a suboptimal one for T2 lesions and a significant failure rate in those cases accentuated by impaired vocal cord mobility (T2b). Surgical salvage provided acceptable rates for ultimate control, but the high percentage of total laryngectomies is disappointing with regard to vocal function. On the basis of the observed results, it would be justified to increase the prescribed radiation doses for all T2 glottic carcinomas to 66 to 70 Gy, which recent reports have shown to provide local control rates of 70% to 80%. Since, however, the application of transoral laser-assisted resection provided encouraging results for T2 glottic cancers, comparable to those with irradiation or conventional surgery, a prospective study to establish the effectiveness of endoscopic laser surgery in T2 carcinoma of the glottis is in progress, including also lesions with impaired vocal cord mobility (T2b). Preoperative and postoperative evaluation of vocal cord mobility and voice quality will be critical. Nevertheless, it should be made clear that this technique requires broad endoscopic and laser surgical skills in order to determine the borders of a tumor and effectively excise it.

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Ann Otol Rhinol Laryngol 105:1996

SEMON'S LAW REVISITED: AN ELECTROMYOGRAPHIC ANALYSIS OF LARYNGEAL SYNKINESIS

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Felix Semon's fascination with vocal fold paralysis led to his theory attributing medialization to isolated abductor paralysis. Later, the Wagner-Grossman hypothesis explained the vocal fold position on the basis of cricothyroid muscle activity. Other authors since that time have dispelled the notion of a role for the cricothyroid muscle in vocal fold position with paralysis. Utilizing laryngeal electromyography, several authors have found electrical activity suggestive of variable reinnervation and synkinesis. This current study included patients with persistent vocal fold immobility. Fourteen patients were studied: half with good voice and half with poor voice. The patients with good voice had a relatively normal arytenoid position and evidence of synkinesis. The group with poor voice had an arytenoid that tipped into the laryngeal inlet and had denervation or poor reinnervation. This suggests that the medialization and upright position of the arytenoid is due to synkinesis.

KEY WORDS — arytenoid, Felix Semon, laryngeal electromyography, synkinesis, vocal fold paralysis.

INTRODUCTION

Throughout the history of laryngology, investigators have studied and compiled theories about the position of the vocal fold in cases of unilateral paralysis, the physiologic basis for the fold position and functional deficit, and the prognosis or likelihood for recovery. Felix Semon was fascinated by his clinical observation of patients with vocal fold paralysis. He asked probing questions and theorized answers. In his "Clinical Remarks," published in 1881, Semon asked the question about vocal fold paralysis "Why does the phonatory position of the vocal cords continue in the later stages? Why do we see during life, or find at the post-mortem table, evidence of retrogressive changes in the posterior crico-arytenoid muscles only..."[1(p220)] The answer Semon offered for the paramedian position of the vocal fold in cases of paralysis was that the adductor centers have "mutual cooperation and anatomical connections...effectively protecting them against disease-producing influences," which he felt were not present in the "unsupported centers of the abductors."[1(p219)] He also felt that the abductor function was "much more automatic than that of the antagonists..." and therefore had less "resistance against disease-producing causes."[1(p219)] Semon accurately described the position of the vocal folds in patients with good voice. He described the paralyzed fold to be in the "cadaveric position," but said that the "contraction of the adductors of the healthy side...is so excessive, when phonation is attempted that the healthy cord crosses the median line to join its diseased fellow."[1(p219)]

Jackson and Jackson[2] agreed with Semon's observation, and stated that with "any lesion of the recurrent nerve...the abductor muscles are affected first, then the tensors second, and the adductors last."[2(p283)] They state that "it is extremely valuable for the maintenance of muscular tone and the prevention of atrophy."[2(p286)] They suggested the "best method is to cause the muscle to jerk by the slowly interrupted galvanic current, placing one electrode in the hands of the patient and touching the mucosa over the region of the affected muscle or muscles."[2(p286-7)]

The Wagner-Grossman hypothesis was developed to explain the vocal fold phenomena related to paralysis because Semon's explanation lacked substantiation. The Wagner-Grossman hypothesis explained the paramedian position of the paralyzed vocal fold on the basis of the action of the ipsilateral cricothyroid (CT) muscle. Dedo, in 1970, reconfirmed this theory on the basis of a laryngeal electromyography (EMG) report of 52 patients with vocal fold paralysis.[3,4]

Since the work of Faaborg-Anderson[5] in 1957 on the normal electrophysiology of the larynx, many laryngologists have concentrated on diagnosis by EMG. Hirano et al[6] used laryngeal EMG to try to determine prognosis in patients with vocal fold paralysis. The onset of electrical activity before 6 months from time of injury was favorable for recovery. Hirano et al believed that after 6 months, the presence of electrical activity was from synkinetic activity.

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Presented at the meeting of the American Laryngological Association, Palm Desert, California, April 29-30, 1995.

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PATIENTS AND FINDINGS

Patient	*Age*	*Sex*	*Cause of Paralysis*	*Voice Quality*	*EMG Findings*	*Endoscopic Findings*
1	76	M	Status post thyroidectomy; RLN resection	Good	Synkinetic TA and PCA; decreased amplitude of MUPs; normal CT activity	VF paramedian; normal arytenoid position
2	65	M	Idiopathic	Good	Synkinetic TA and PCA; decreased amplitude of MUPs; normal CT activity	VF paramedian; normal arytenoid position
3	55	M	Status post mediastinoscopy; RLN injury	Good	Synkinetic TA and PCA; decreased amplitude of MUPs; normal CT activity	VF paramedian; normal arytenoid position; slightly irregular motion on phonation with stroboscopy
4	59	F	Status post thyroidectomy; RLN injury	Good	Synkinetic TA and PCA; normal amplitude of MUPs; normal CT activity	VF paramedian; normal arytenoid position; slightly irregular motion on phonation with stroboscopy
5	42	F	Idiopathic	Good	Synkinetic TA, CT, and PCA; decreased amplitude of MUPs	VF paramedian; normal arytenoid position; slightly irregular motion on phonation with stroboscopy
6	56	F	Status post resection of vagal nerve; schwannoma	Good	Synkinetic CT, TA, and PCA; decreased interference pattern; decreased amplitude of MUPs	VF paramedian; normal arytenoid position; jerky, irregular motion on stroboscopy
7	81	F	Status post CABG; RLN injury	Good	Synkinetic TA and PCA; decreased amplitude of MUPs; normal CT activity	VF paramedian; normal arytenoid position; jerky, irregular motion on stroboscopy
8	62	M	Esophageal carcinoma	Poor	Few fibrillations; no voluntary activity or MUPs; normal CT activity	VF paramedian; bowed; anteriorly and medially tipped arytenoid
9	54	M	Status post thymoma resection	Poor	Occasional fibrillations; no voluntary activity; occasional complex repetitive discharge; normal CT activity	VF intermediate position; anteriorly displaced arytenoid
10	84	M	Status post thyroidectomy; RLN resection	Poor	Fibrillations; some positive waves; no MUPs; normal CT activity	VF intermediate; anteriorly tipped arytenoid
11	45	F	Status post vagal schwannoma excision	Poor	Few MUPs; occasional polyphasics; markedly reduced interference pattern	VF paramedian; medially tipped arytenoid
12	63	F	Lung carcinoma	Poor	Fibrillations; no voluntary activity; normal CT activity	VF paramedian; anteriorly and medially tipped arytenoid
13	52	F	Idiopathic	Poor	Markedly decreased amplitude and numbers of potentials; few complex polyphasics	VF intermediate; anteriorly and medially tipped arytenoid
14	73	F	Idiopathic	Poor	Synkinetic activity of TA, CT, and PCA; markedly reduced numbers and amplitude of MUPs; markedly reduced interference pattern	VF paramedian; anteriorly tipped arytenoid

EMG — electromyography, RLN — recurrent laryngeal nerve, TA — thyroarytenoid, PCA — posterior cricoarytenoid, MUPs — motor unit potentials, CT — cricothyroid, VF — vocal fold, CABG — coronary artery bypass graft.

Hiroto et al,[7] in a study of patients, also found electrical potentials in immobile folds suggesting the presence of synkinesis.

Benninger et al,[8] in their review of the unilateral paralyzed vocal fold, described the position of the arytenoids as related to the relative balance of muscle contractions that control the arytenoid position. They said that injury to the lateral cricoarytenoid (LCA) innervation can result in the arytenoid's being partially unsupported, and therefore often falling medially into the supraglottic inlet. Posterior cricoarytenoid

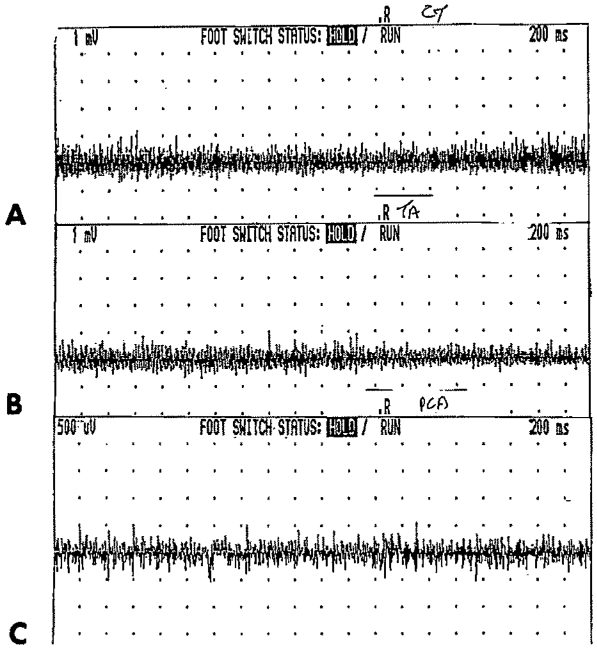
A
B
C
,R CT
1 mV
FOOT SWITCH STATUS: HOLD / RUN
200 ms
,R TA
1 mV
FOOT SWITCH STATUS: HOLD / RUN
200 ms
,R PCA
500 uV
FOOT SWITCH STATUS: HOLD / RUN
200 ms

Fig 1. Simultaneous electromyography of A) cricothyroid, B) thyroarytenoid, and C) posterior cricoarytenoid muscles in patient with good voice (synkinesis).

(PCA) loss decreases lateral sliding and abduction. Thyroarytenoid (TA) loss causes atrophy of the vocal fold with bowing due to loss of muscle mass, and lack of anterior pull on the vocalis process. The paralyzed fold opens earlier in the glottic wave and is missing the normal undulating wave.

Crumley[9] described the voice during the acute phase of denervation as breathy, hoarse, and often diplophonic. Patients may also have some aspiration while drinking liquids. With the onset of the intermediate phase (3 to 4 weeks), there is usually only slight or no aspiration with liquids, and some improvement in voice quality. This change may be related to improvement in electrical activity.

This study was undertaken to evaluate the effect of variable innervation and synkinesis on the position of the vocal fold and arytenoid and the quality of voice.

METHODS AND MATERIALS

Fourteen patients (see Table) who had a unilateral vocal fold paralysis for greater than 1 year were included in this study. Patients whose vocal fold paralysis was due to a cerebrovascular accident, brain stem injury, or skull base injury to the vagus nerve were not included in this study, since these injuries would add many other uncontrolled parameters such as sensory deprivation, altered reflexes, and pharyngoesophageal weakness. The group consisted of 7 patients who regained good vocalization despite a persistent vocal fold immobility, and 7 patients who continued to have poor voice with vocal fold immobility. The group consisted of 6 men (3 with good voice and 3 with poor voice) and 8 women (4 with good voice and 4 with poor voice). The ages ranged from 42 to 84, with a mean of 62 years in both groups. The cause of the vocal fold paralysis was peripheral nerve injury, except in 4 patients with an idiopathic nerve palsy.

All the patients had a careful history taken for cause and possible other nerve weakness. Patients with other nerve palsies or more global central disease were excluded from the study. Patients who had a vocal fold paralysis who went on to recovery of function were excluded. All patients had both nasal fiberoptic and oral rigid laryngostroboscopy, with the fold characteristics such as function, position, muscle mass, fold shape, and arytenoid position noted.

All of the patients then had laryngeal EMG with monopolar needle and monopolar hooked-wire electrodes. The TA, CT, and PCA muscles were tested. The PCA muscles were studied by manually rotating the larynx and impaling the PCA muscle with a percutaneous puncture posterior to the thyroid lamina, by means of a technique we previously have described.[10] The studies were carried out with a Nicolet Viking model. Single muscle and simultaneous recordings were taken. The waveform morphology, size of the motor unit potentials (MUPs), and recruitment pattern were then analyzed for each patient. The electrode position was verified by fiberoptic endoscopy when necessary, and by functional verification. The EMG electrodes were placed in the muscle, obtaining crisp-sounding electrical activity, and except for fibrillation potentials, any activity of less than 150 µV was excluded to avoid confusion with background noise of adjacent muscle signal. The patients were asked to swallow, phonate, and sniff to activate the laryngeal muscles and identify the pharynx. Synkinesis was determined when a dense interference pattern of MUPs was found with greater than 150-µV amplitude in the CT, TA, and PCA muscles during phonation and sniffing.

RESULTS

An important observation on stroboscopic examination of these patients was that all of the patients who had good voice had their immobile vocal fold in the paramedian position with the arytenoid sitting upright, at the same level as the contralateral, functional side. Two of them had slightly irregular or jerky motion of the TA muscle on phonation. On EMG, all of these patients with good voice had evidence of synkinetic activity in the TA and PCA muscles (Fig 1). All of these patients had MUPs with

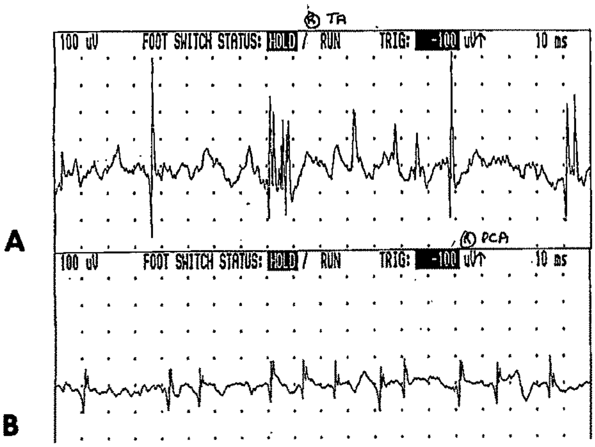

Fig 2. Simultaneous electromyography of A) thyroarytenoid and B) posterior cricoarytenoid muscles in patient with poor voice (giant and polyphasic waves in thyroarytenoid; complex repetitive waves in posterior cricoarytenoid).

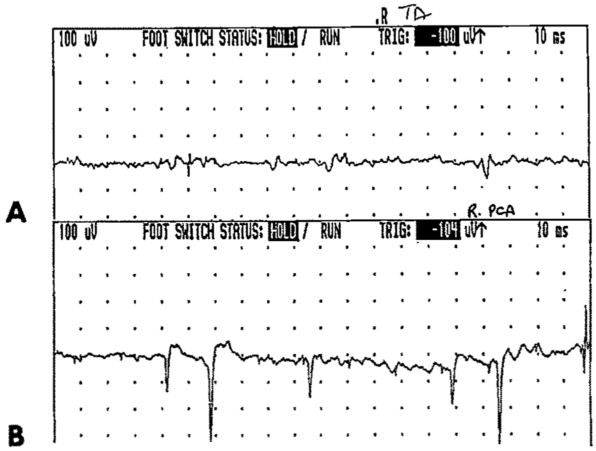

Fig 3. Simultaneous electromyography of A) thyroarytenoid and B) posterior cricoarytenoid muscles in patient with poor voice (fibrillation potentials in thyroarytenoid; positive waves in posterior cricoarytenoid).

decreased amplitude as compared to the normally functioning side. Five of the 7 had normal CT activity. One patient with an idiopathic disorder and 1 patient with a schwannoma had synkinetic activity displayed in the CT muscle as well.

Analysis of the group of patients with poor voice showed 4 patients with a vocal fold in the paramedian position, and 3 with an intermediate position. In all 7 patients, the position of the arytenoid was abnormal. Three of these patients had an anteriorly tipped arytenoid, 3 had anterior and medial displacement, and 1 had a medially displaced arytenoid. This abnormal arytenoid position left the vocal fold in an unstretched position, with 1 of the 7 showing frank bowing. On EMG, 4 of the 7 displayed fibrillation potentials, with no voluntary activity (Figs 2 and 3). Four of the patients had normal CT activity. One patient who had an idiopathic cause of immobility displayed synkinetic activity in the TA, CT, and PCA muscles, but had marked reduction in the numbers and amplitude of MUPs, with a reduced interference pattern. Two of the patients had small, complex polyphasics, with significantly decreased amplitude and numbers of MUPs.

DISCUSSION

Synkinesis is defined as the unintentional movement accompanying a volitional movement. This synchronous contraction of muscles that usually have independent neural stimulation is thought to be related to misdirected and inappropriate reinnervation of muscles. Perhaps a better term would be simultaneous isometric contraction of antagonistic muscles (SICAM). Crumley and McCabe,[11] in 1982, performed recurrent laryngeal nerve (RLN) section in dogs, and found that the reinnervation was unpredictable and random. The branch of the RLN to the PCA muscle (abductor fibers) is mostly slow-twitch fibers, whereas the fibers to the TA and LCA muscles are mostly fast-twitch. Slow-twitch fibers have been shown to reinnervate many more muscle fibers than fast-twitch fibers.

Hiroto et al observed, during an EMG investigation of patients with vocal fold paralysis, that the earliest neuromuscular electrical potentials were found 2 months after the RLN injury. The authors found in 9 patients studied "unusual patterns of electrical activity."[7(p301)] Electrical activity was found in the TA muscle on inspiration and phonation. They suggested that "this unusual pattern resulted from a misdirection of regenerating nerve fibers."[7(p301)] There was also a decrease in the amplitude and numbers of MUPs, as well as a reduced interference pattern.

If the normal vocal fold activity does not return, the quality of the voice production will be directly related to the position of the vocal fold; the tone, configuration, and muscle mass of the TA muscle; and the compensatory activity of the contralateral muscles. The Semon and Wagner-Grossman hypotheses tried to explain the fold position on the basis of selective abductor weakness; the interarytenoid muscle pull; or the degree of activity of the CT muscle. Woodson[12] reported on vocal fold position in 14 cases of unilateral paralysis (7 vagal and 7 RLN) and noted no difference in the vocal fold position. She suggested that the fold position was related to some spontaneous reinnervation, and not CT activity, as suggested by Wagner-Grossman. Woodson's[13] animal studies also demonstrated that variability in the vocal fold position was due to random reinnervation.

Hirano et al,[6] in a study of 114 patients with vocal fold paralysis based on RLN paralysis or RLN plus superior laryngeal nerve paralysis, found that the vocal folds remained paramedian rather than intermediate, as suggested by the Wagner-Grossman hypothesis. Hirano et al also noted that as an alternative to rotation, there may be an anterior displacement of the paralyzed vocal fold with a shortening of the fold. Tanaka et al[14] also noted that the CT activity had little to do with vocal fold bowing. When laryngeal EMG were performed on patients with vocal fold paralysis and bowing, 69% had markedly reduced TA activity.

Koufman et al recently published a paper that also analyzed vocal fold position in a number of different disorders, and they concluded that the "cricothyroid muscle does not influence the vocal fold in laryngeal paralysis."[4(p368)] The authors performed laryngeal EMG of the TA muscles and had patients sniff (maximal abduction). They found "paradoxical recruitment." Koufman et al state that "it is likely that different patterns of spontaneous aberrant regeneration might be the explanation for different vocal fold positions after laryngeal paralysis."[4(p371)] Depending on the nature of the injury, reinnervation could come from nerves adjacent to the RLN. Reinnervation can come from the ansa cervicalis nerves to the strap muscles, or from other local nerve supplies. Nomoto et al,[15] in a study of denervated feline laryngeal muscles, found nerve terminals, resembling autonomic nerves, reinnervating all neuromuscular junctions.

Most EMG studies of patients with vocal fold paralysis were performed to determine the likelihood of recovery of normal function. A number of studies found recovery of electrical activity, but no vocal fold motion. The frequency of laryngeal synkinesis after RLN injury is unknown.[16] Some of the patients who have been classified as having long-term vocal fold paralysis in previous reports actually have an immobile fold with mixed reinnervation and synkinesis. The current study is to determine, with laryngeal EMG, whether patients who achieve good voice but continued vocal fold immobility are different from patients who have poor voice with continued vocal fold immobility. Seven patients in each group were studied, and all patients with good voice were found to have synkinetic activity (SICAM) of the TA and PCA muscles. Two patients additionally had synkinetic activity in the CT muscle. The vocal folds exhibited normal shape, muscle mass, and arytenoid position. This left the vocal fold in a paramedian position with good tone and glottic wave. Two of the patients had a slight tremor or jerky motion of the immobile vocal fold when phonating, suggesting an alternating imbalance between antagonistic muscles.

In contradistinction, most of the patients who displayed persistent poor voice had signs of chronic denervation of the TA and PCA muscles. Two of the 7 patients with poor voice had some signs of reinnervation (occasional polyphasics or complex giant waves) but had a markedly decreased number and amplitude of MUPs, as well as a markedly reduced interference pattern. This finding suggested that although there was some reinnervation, the amount of electrical activity was so small that the muscles were effectively denervated. The vocal folds often assumed an intermediate position with an open glottic chink. The arytenoids were tipped anteriorly and medially, causing a shortening of the TA muscle, allowing some bowing. This further increased the breathiness of vocalizations. These arytenoid positions are consistent with the findings of Hirano et al.[6] The change in position of the arytenoid with a lax, bowed vocal fold may explain why some patients continued to have poor glottal waves with vocal cord paralysis.

Kokesh et al[17] correlated the stroboscopic pattern with EMG in patients with vocal fold paralysis. They found that 8 of 10 patients with EMG evidence of reinnervation or partial denervation had good symmetric mucosal waves, whereas 3 of 10 with denervation had symmetric mucosal waves. Sercarz et al[18] studied 3 volunteers who had SLN and RLN blocks to simulate a vocal fold paralysis, and 20 patients with a unilateral paralyzed vocal fold, with videostroboscopy. Kokesh et al found that the "most significant finding was asymmetry of the traveling wave motion."[17(p855)] The normal vocal fold had a faster wave that caused a phase difference between the vocal folds. In our present study, most of the patients with good voice and an immobile fold had a symmetric glottal wave, most likely representing good muscle tone due to aberrant regeneration.

CONCLUSIONS

The observations of vocal fold position and function made by Semon are unlikely to be explained only by abductor fiber weakness, or adductor predominance. It is similarly unlikely to be caused by CT involvement, as suggested by the Wagner-Grossman hypothesis. In concurrence with the work of a number of other investigators, our current study suggests that the cause is variable reinnervation and synkinesis or simultaneous contraction of antagonistic muscles (SICAM). These variable changes in neuromuscular activity will dictate the fold position, the arytenoid position, and the vocal fold tone, mass, and mucosal wave. These attributes will translate into the functional characteristics observed.

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Ann Otol Rhinol Laryngol 105:1996

BENIGN PAROXYSMAL POSITIONAL VERTIGO OF POSTERIOR SEMICIRCULAR CANAL: RESULTS IN 160 CASES TREATED WITH SEMONT'S MANEUVER

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One hundred sixty patients affected by benign paroxysmal positional vertigo of the posterior semicircular canal were treated with Semont's maneuver. Complete recovery after only one session was achieved in more than 50% of cases. After a maximum of five sessions, almost all patients had recovered; only 8 needed another kind of rehabilitation. No drug therapy was required for any patient.

KEY WORDS — benign paroxysmal positional vertigo, Semont's maneuver.

INTRODUCTION

The basic characteristics of benign paroxysmal positional vertigo (BPPV) are implicit in the name. Fundamentally an episodic vertigo of the objective rotational type, it is sometimes associated with a feeling of "inversion" or "levitation." It is defined as "typical" if the clinical and laboratory data coincide only with disturbances of the semicircular canals; it is considered "atypical" if the characteristics of evocation are not standard or are accompanied by otoneurologic alterations or more general disturbances of the internal ear or of the central vestibular pathways. The typical form, with its paroxysmal crisis of brief duration (with rapidly increasing vertigo and a stable phase followed by regression), may occur several times on the same day or on those following.

Symptoms are triggered by a certain head position (rarely more than one) that the patient can often describe precisely and that can be reproduced artificially. Even presupposing spontaneous recovery, not all authors agree the condition is benign, since patients sometimes report symptoms lasting for many years, even if accompanied by more or less long periods of good health. On the basis of observations by Barany[1] first and others later,[2-4] Schuknecht[5] developed a clinical classification of BPPV based on the otoconial hypothesis. According to this theory, fragments of otoconia detach from the utricular macula and enter into contact with the cupula of the posterior semicircular canal (PSC), transforming it into a sensor of linear acceleration. When the patient moves into the crucial position, the force of gravity acts on the otoconial mass and determines an outward flow of fragments. According to the laws of Ewald, this receptor stimulation evokes a vestibular-oculomotor reflex (VOR) on the vertical plane of the PSC, which is manifested clinically as a nystagmus (NY), sometimes clearly described as vertical or purely rotatory (counterclockwise in involvement of the right PSC, clockwise in involvement of the left PSC) or even vertical-rotatory.

Benign paroxysmal positional vertigo may result from canalithiasis rather than cupulolithiasis.[6-10] This theory holds that fragments of amorphous material, probably of otoconial origin, are suspended in the PSC endolymph. However, the only in vivo evidence of these fragments is provided by the Parnes and McClure[11] observations made during PSC occlusion surgery in patients with BPPV who were refractory to physical treatment. When the head is moved into the triggering position, the fragments sink into the most dependent position in the canal, causing the endolymph to move away from the ampulla and overcome the cupula inertia, resulting in inappropriate stimulation of the canal ampullary nerve.

Whatever the etiopathogenic origin, the pattern of NY in a typical attack of BPPV is always the same.

1. Latency in onset reflects the time necessary for the gravitational push to put the otoconial debris in motion.

2. The NY increase-plateau-decrease pattern results in endolymph inertia versus the stimulus caused by the ampullar crest maneuver.

3. Nystagmus inversion upon returning to the initial position (seated) is almost always of minor importance, and is sometimes only a subjective feeling of vertigo.

4. Nystagmus exhaustion (ie, the tendency to alle-

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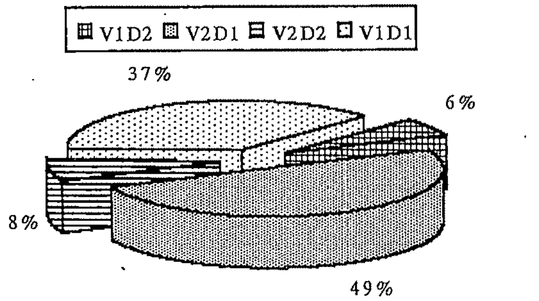


Fig 1. Initial self-evaluation (see Table). V1 — light dizziness, V2 — vertigo, D1 — duration of less than 1 minute, D2 — duration of less than 15 minutes.

viation until disappearance if the crucial head position is resumed repeatedly at short intervals) can be explained because the position generates an endolymphatic flow that tends to disentangle the otoliths in the ampullar crest or the long arm of the PSC. If the otoliths have enough time to return to their original position, before the crucial position is resumed, the same vertigo effect and NY will be caused again. Instead, in repetitions after a brief passage of time, the vertigo will be less intense, and the NY will have a longer latency time and minor duration. With a quick series of stimuli, symptoms gradually decrease until they disappear, because for every action some otoliths will detach and will be unable to drift onto the cupola.

This description is valid for BPPV of the PSC — the most frequent because of the anatomic position of the canal with respect to the utricular macula — but BPPV of the lateral canal has also been described,[12-14] in isolation or associated with BPPV of the PSC or consequent to rehabilitative maneuvers. The trigger is the action of turning onto one side, which induces a very violent horizontal geotropic NY with latency — the increase-plateau-decrease pattern followed by NY inversion. The same clinical picture occurs less intensely on the other side, and often in these patients the Dix-Hallpike maneuver also provokes a similar NY. The position of the lateral semicircular canal (LSC) makes one think of a pathogenic mechanism similar to BPPV of the PSC. Endolymphatic flow generated from the maneuver appears to be determined by the speedy dislocation of a dense mass (otolithic fragments) located in the nonampullar end of the LSC or by changes in endolymph viscosity in the LSC that make it more susceptible to variations in the direction of gravity.[13] Therefore, BPPV in the LSC is usually considered together with, and not separate from, BPPV in the PSC, as both forms are due to increased semicircular canal sensitivity to the force of gravity. The elastic properties of endolymphatic fluid, and the tendency of fragments to disperse, end the stimulus, the vertigo, and the NY.

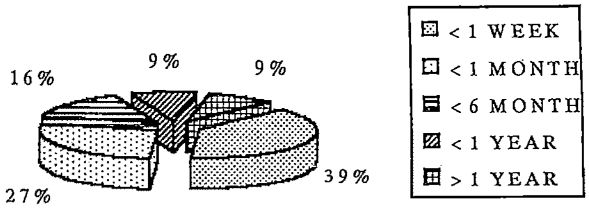


Fig 2. Duration of symptoms.

Nystagmus inversion after a certain time may be due to a central mechanism or to the reflux of the otoliths and the endolymph fluid in the LSC.

Occasionally, BPPV may be caused by cranial trauma, impairment of blood circulation in the internal ear, labyrinthitis such as in Meniere's disease, toxic labyrinthitis (drugs), middle ear surgery, general illnesses like macroglobulinemia or hypergammaglobulinemia, prolonged bed rest after surgery,[2,5,15-22] etc. In most cases the pathogenesis remains obscure.[15,17,21,23-29]

The aim of this work was to verify the efficacy of the rehabilitative maneuver of Semont,[30] in cases in which it was utilized alone and in cases in which its failure (after at least five sittings) had required a rehabilitative cycle according to the FIVE technique[31] or the Italian Otorhinolaryngologist Hospital Association (AOOI) protocol.[32]

MATERIALS AND METHODS

One hundred sixty patients (104 women, 56 men, age range 15 to 78 years, mean age 47.1) affected by "typical" BPPV were selected. Ninety-eight patients out of 160 (61%) were suffering from intense vertigo that made them freeze their movements; 62 (39%) had slight vertigo accompanied by good motor activity (Fig 1). The onset of vertigo was within the previous week in 62 patients (39%), month in 43 patients (27%), 6 months in 26 patients (16%), and year in 14 patients (9%) and had occurred more than 1 year earlier in 15 patients (9%). In 1 case vertigo had begun 4 years earlier, after the patient was extracted from a car involved in an accident (Fig 2).

The BPPV was diagnosed on the basis of the following tests: audiometry, Romberg's test, Unterberger's test, observation of spontaneous NY with Frenzel glasses, positional and positioning maneuvers (right hip, left hip, Dix-Hallpike, Rose, Semont diagnostic), hot and cold stimulation according to the Fitzgerald-Hallpike method, impedance tests, evoked brain stem auditory potentials, and neurologic and vascular evaluation. Intensity of vertigo was estimated by a new self-evaluation protocol set up by the Italian Group for the Pharmacological Study of Vertigo (GISFaV; see Table). All tests, except the posi-

PROTOCOL FOR SELF-EVALUATION SET UP BY ITALIAN GROUP FOR PHARMACOLOGICAL STUDY OF VERTIGO (GISFaV)

Evaluation of intensity of vertigo (V)
  1) Light dizziness (the patient can stand up and walk)
  2) Vertigo (the patient must stop during the crisis)
  3) Disabling vertigo (the patient must be always still)
Associated symptoms (N)
  1) Absent
  2) Nausea
  3) Vomiting
Duration of crisis (D)
  1) Less than 1 minute
  2) Less than 15 minutes
  3) A few hours
  4) One or more days
Frequency of crisis (F)
  1) Every month
  2) Every week
  3) Every day
  4) Several times a day
Quality of life (Q)
  0) Normal
  1) Incomplete inactivity
  2) Total inactivity

Modified from protocol GISFaV from 2°Cogresso Nazionale del Gruppo Italiano per la Farmacologia della Vertigine (GISFaV). Florence, Italy: Pacini Editore, 1991.

tional and positioning movements, were negative, thus ruling out that the vertigo was caused by any other disease of the labyrinth. The Semont diagnostic and/or the Dix-Hallpike maneuvers were positive in all cases, with the characteristic torsional NY appearing after a latency period and gradually disappearing. Repeating the maneuver showed NY fatigability.

After diagnosis of BPPV, Semont's maneuver was recommended and all subjects were informed about the side effects. Semont's maneuver[30] is performed as follows: the patient sits on the edge of the bed looking straight ahead. With the physician holding his or her head steady, the patient is rapidly guided down until lying on the affected side. The head is rotated upward into a 45° angle. This rapid change of position elicits the paroxysmal vertigo with torsional NY. The patient remains in this position for 2 minutes or until the NY disappears. The patient is quickly moved onto his or her other side and the head is rotated downward into a 45° angle. This countermaneuver elicits the paroxysmal vertigo; the NY response from the first to the second Semont's position remains the same. After 2 minutes the patient is slowly raised to the seated position with his or her head straight. When positional NY persisted after Semont's maneuver, the maneuver was repeated until disappearance. In the following 24 hours the patient was asked to avoid brisk movements and actions that could provoke rapid acceleration or deceleration in head maneuvers, but to remain mobile. After 2 days, a new subjective estimate using the GISFaV scales was made, and the Dix-Hallpike maneuver was performed. When subjective disturbances or clinical signs (NY) were absent, patients were reassessed after 7 days, 30 days, and 6 months. If symptoms persisted, the patient was subjected to another Semont's maneuver, repeated as needed for a maximum of five times.

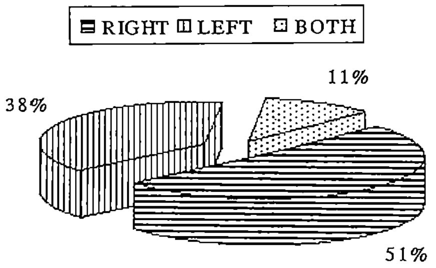


Fig 3. Location of benign paroxysmal positional vertigo.

If these cycles did not produce complete normalization, the patient was treated by a different rehabilitative protocol according to the FIVE[31] or AOOI[32] recommendations.

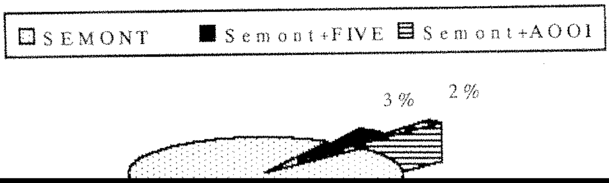
RESULTS

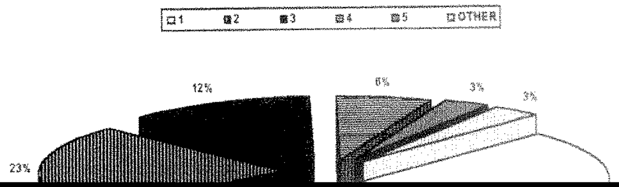
In 82 of the 160 patients (51%) the semicircular canal involved was the right PSC, and in 61 (38%) the left PSC. Both PSCs were involved in only 17 patients (11%; Fig 3).

After all cycles of treatment, none of the subjects presented objective or subjective troubles (V0, N0, D0, F0, Q0) on the GISFaV scale (see Table). In 152 cases, Semont's maneuver alone was enough (95%). In the other 8 (5%), at the end of the five sessions (the maximum number programmed), it was necessary to proceed to an alternative cycle of reeducation according to the FIVE technique (5 cases) or the AOOI protocol (3 cases) because of the persistence of vertigo symptoms — which were, however, much less intense than before therapy with Semont's maneuver (Fig 4). After 6 months no patient presented signs or symptoms of vertigo.

Of the 152 patients treated only with Semont's maneuver, disturbances disappeared in 82 after the first treatment (53% of the 160), in 36 after the second (23%), in 19 after the third (12%), in 10 after the fourth (6%), and in 5 after the fifth (3%; Fig 5).

The success rate was linked to the time of onset. One treatment session was sufficient in about 95% of patients presenting symptoms for less than 1 week, in 44% of patients with symptoms for less than 1 month,





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The 100% success rate that we achieved using Semont's maneuver alone (95%) or followed by rehabilitation therapy (5%) and the success rates reported elsewhere for other maneuvers[7] show that surgery should not be recommended in BPPV, particularly when one considers the risks inherent in any operation and the specific, although rarely observed, complications in the inner ear. Surgery might be recommended only for those very few cases of BPPV (perhaps not really typical?) that are refractory to both liberating maneuvers and rehabilitation, none of which were observed in this study.

ACKNOWLEDGMENT — The authors thank Dr G. A. Boyd for the translation of this paper.

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Ann Otol Rhinol Laryngol 105:1996

DIMENSIONS OF THE SINUS TYMPANI AND ITS SURGICAL ACCESS VIA A RETROFACIAL APPROACH

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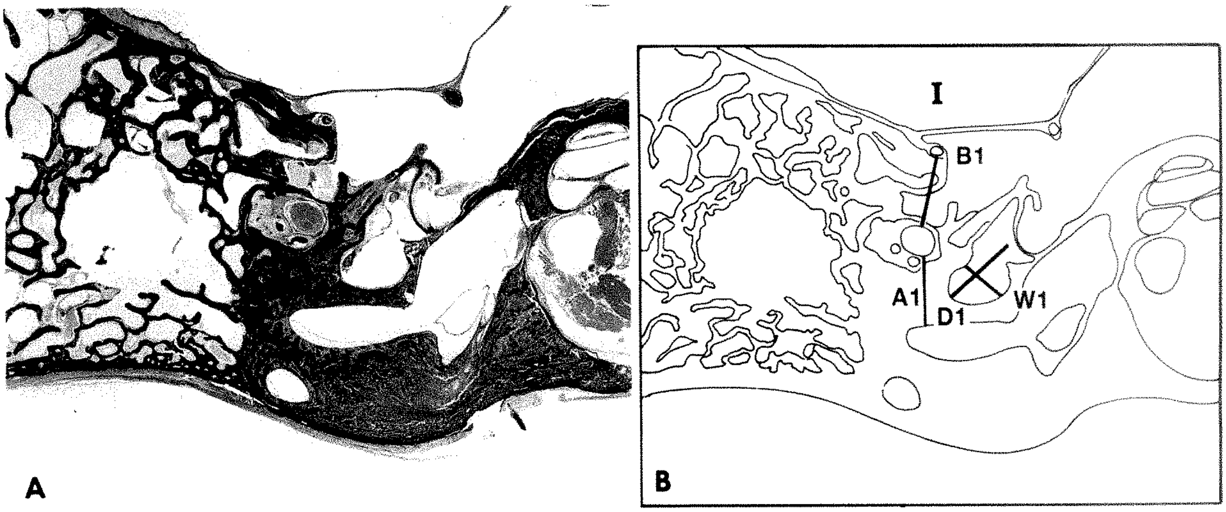

Fig 1. Human temporal bone at level of stapedial tendon (level I). A) Horizontal section. B) Tracing of same section indicating topographic measures A, B, W, and D. A — facial nerve–lateral semicircular canal (FN-LSC) interspace, B — facial nerve–chorda tympani (FN-ChT) interspace, W — sinus tympani (ST) width, D — ST depth.

ated and used in this study only if available sections included the stapedial tendon, round window, PSC, LSC, FN, and chorda tympani (ChT). Any temporal bone specimens with artifacts, poor staining, or failure to include the structures of interest were excluded from the study.

An otomicroscope was fitted with a video camera, and the image of each temporal bone section was transmitted to an Apple Macintosh computer screen. Precise measurements were made after scaling the distances on the screen with the digital image processing and analysis software package Image 1.43.

Anatomic topography was quantified by measuring the shortest distance between the FN epineurium and the PSC (A) and the shortest distance between the FN epineurium and the ChT (B). The maximum width (W) and the maximum depth (D) of the ST were also measured. The depth of the ST was measured as the maximum distance from the midpoint of the entrance to the ST to its most posterior extent. Figures 1 through 4 are photomicrographs accompanied by line drawings of temporal bone sections through each of the four levels from which topographic distances A, B, W, and D were measured. These measurements were taken at four levels within each temporal bone: at the level of the stapedial tendon (level I), midlevel between the stapedial tendon and the round window (level II), at the round window (level III), and in the hypotympanum (level IV) (Fig 5). The thickness of the compact bony plate separating the ST from the mastoid air cells (E2) and

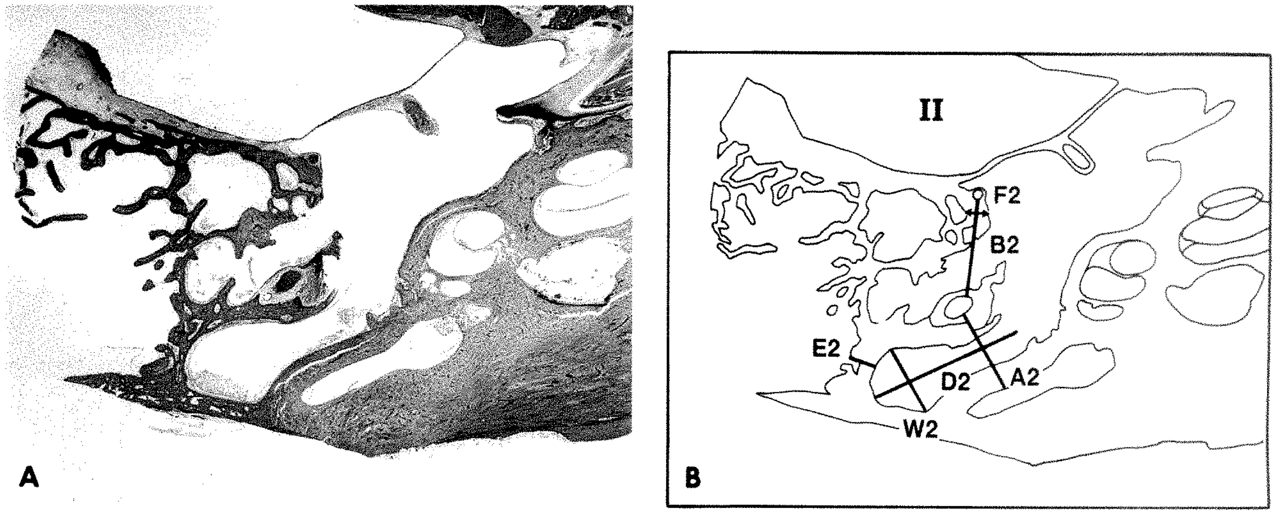

Fig 2. Human temporal bone midway between level of stapedial tendon and round window (level II). A) Horizontal section. B) Tracing of same section indicating topographic measures A, B, W, D, E2, and F2. A — FN–posterior semicircular canal (PSC) interspace, B — FN-ChT interspace, W — ST width, D — ST depth, E2 — ST bone plate thickness, F2 — facial recess (FR) bone plate thickness.

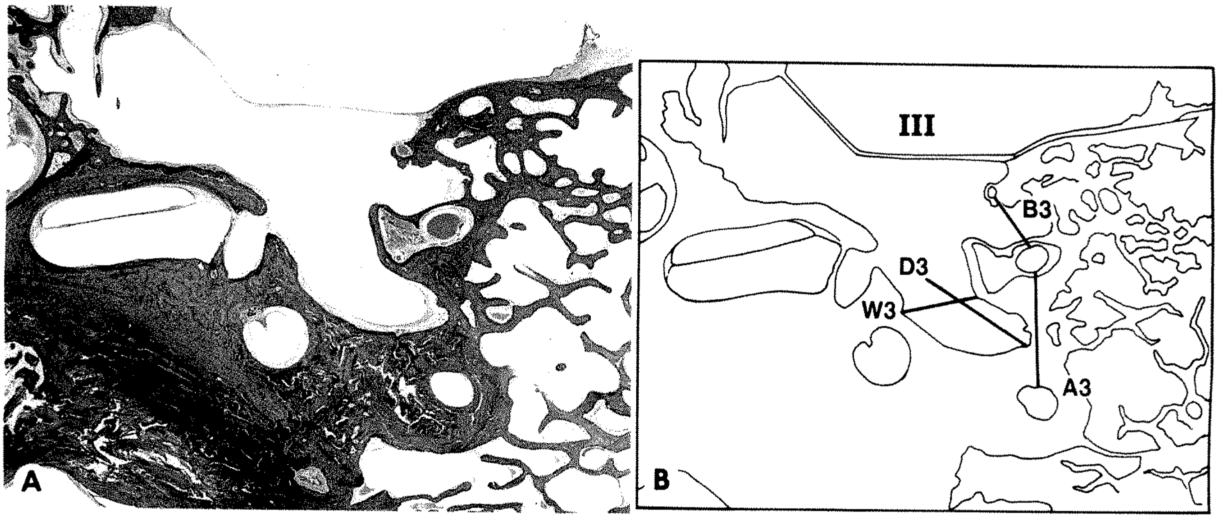

Fig 3. Human temporal bone at level of round window (level III). A) Horizontal section. B) Tracing of same section indicating topographic measures A, B, W, and D. A — FN-PSC interspace, B — FN-ChT interspace, W — ST width, D — ST depth.

the bone separating the facial recess from the mastoid air cells (F2) was measured only at level II, between the ST and the round window niche. Table 1 organizes the 18 measurements taken from each of the temporal bone specimens.

The degree of aeration in the compact bone of the posterior walls of both the ST and the facial recess (FR) was assessed and the bone was categorized as pneumatized or acellular. Partially pneumatized bones were included in the pneumatized group. Table 2 shows the distribution of pneumatization observed within three areas of the temporal bones — in the region of the ST, in the region of the FR, and in the mastoid portion of the temporal bone.

The data were analyzed for effect of side, gender, degree of pneumatization in the regions of the ST and the FR, and level within each specimen. Because there were 327 bones from 323 subjects, a four-factor repeated-measures analysis of variance (ANOVA) approach for unbalanced data was used. The ANOVA model used to compute the effect of side (right or left temporal bone) was a mixed multivariate model that included a random effect for subject. The purpose of this analysis strategy was to isolate the effect of subject on bone characteristics and separate it mathematically from the contributions of the other terms such as side, level, gender, and pneumatization. The resulting simultaneous solution is adjusted for all terms in the model, allowing the use of both temporal bones from the same subject. Computations were performed by the SAS General Linear Models proce-

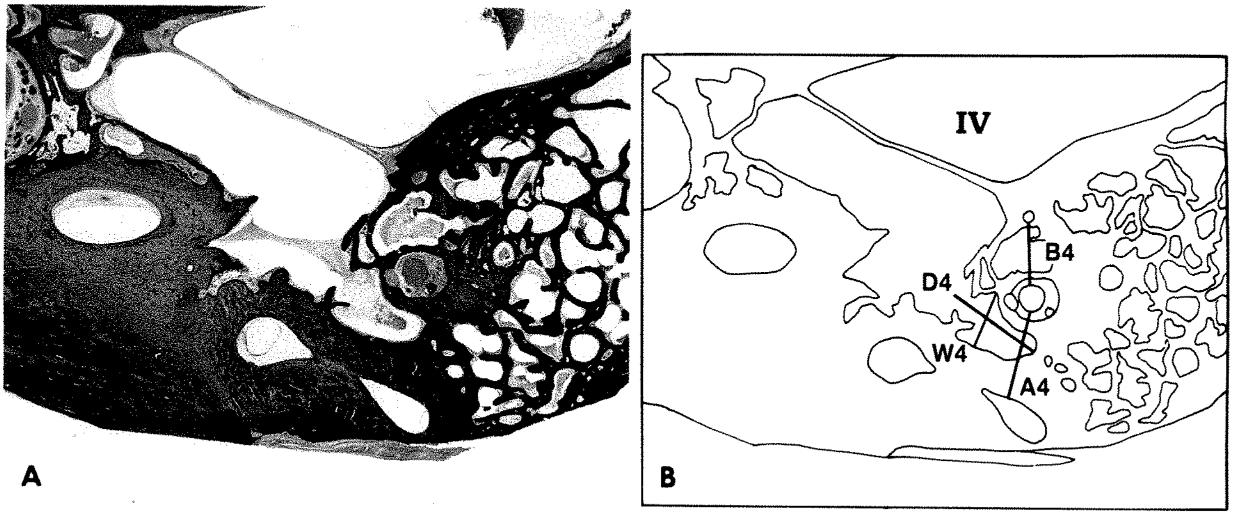

Fig 4. Human temporal bone at level of hypotympanum (level IV). A) Horizontal section. B) Tracing of same section indicating topographic measures A, B, W, and D. A — FN-PSC interspace, B — FN-ChT interspace, W — ST width, D — ST depth.

TABLE 1. TOPOGRAPHY REFERENCE TABLE OF DISTANCES MEASURED AT FOUR STANDARDIZED LEVELS

Level	*FN-LSC or FN-PSC*	*FN-ChT*	*ST Width*	*ST Depth*	*Thickness of ST Bone Plate*	*Thickness of FR Bone Plate*
I — Stapedial tendon	A1	B1	W1	D1		
II — Between stapedial tendon and round window	A2	B2	W2	D2	E2	F2
III — Round window	A3	B3	W3	D3		
IV — Hypotympanum	A4	B4	W4	D4		

FN — facial nerve epineurium, LSC — lateral semicircular canal, PSC — posterior semicircular canal, ChT — chorda tympani, ST — sinus tympani, FR — facial recess.

dure version 6.08.[4]

RESULTS

Table 3 summarizes the topographic measures by site and level. A critical distance reflecting the accessibility of the ST from the mastoid is the distance between the FN epineurium and the LSC at level I, and between the FN epineurium and the PSC at levels II, III, and IV. The average distance between these structures increased from 2.33 to 3.78 mm with progressively inferior sections. These differences in interspace dimensions were all significantly different by ANOVA except at level II to level III. The maximum distance between the FN epineurium and the labyrinth was 3.78 mm and this was at the level of the hypotympanum (level IV) inferior to the ST.

In comparison, the traditional access route to the middle ear from the mastoid is via the FR and is limited by the distance between the FN epineurium and the ChT. In the current series of temporal bones examined, the maximal distance between the FN and the ChT was at level I, averaging 3.04 mm in the region of the stapedial tendon. In contrast to the ST topography, the FN-ChT distance decreased with successive inferior sections to a gap of 2.33 mm at level III.

A comparison of the distances between critical structures delimiting the FR and the ST indicates that accessibility to the mesotympanum from either approach is highly dependent on the level examined. At the level of the stapedial tendon, the FN-ChT interspace was larger than the FN-LSC interspace: 3.04 versus 2.33 mm. However, at all subsequent levels, the FN-PSC interspace was consistently and significantly larger in size than the FN-ChT interspace. At level II the FN-PSC interspace was 2.86 mm, versus 2.68 for the FR. At level III the FN-PSC interspace was 2.98 mm, versus 2.33 for the FR. The size dimensions of the ST varied, with an average W of 1.49 ± 0.39 (SD) mm and a range of 0.49 to 3.87 mm, and an average D of 2.06 ± 0.60 mm and a range of 0.2 to 9.9 mm.

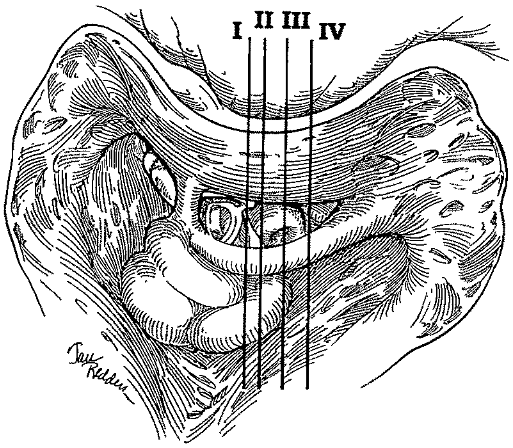

Fig 5. Surgical view of mastoid and middle ear and four standardized levels at which measurements were taken.

Effect of Side and Gender. One hundred sixty-nine right and 158 left ears were examined. One hundred seventy-four bones were from males and 153 were from females. None of the topographic measures showed evidence of sidedness. Likewise, there were no significant differences for any of the anatomic measures when examined for the effect of gender.

Effect of Pneumatization. The mastoid cavity was pneumatized in 321 cases and partially pneumatized in 6 cases. There were no sclerotic mastoids in this series. The compact bone between the ST and mastoid air cells was pneumatized in 20 cases, partially pneumatized in 56 cases, and acellular in 251 cases. The bone plate between the FR and the mastoid was pneumatized in 100 cases, partially pneumatized in 128 cases, and acellular in 99 cases (Table 2).

The pneumatized and partially pneumatized specimens were combined and contrasted with the acellu-

TABLE 2. DEGREE OF PNEUMATIZATION NOTED IN THREE LOCATIONS WITHIN TEMPORAL BONE

	Pneumatized	*Partially Pneumatized*	*Sclerotic*
Sinus tympani	20	56	251
Facial recess	100	128	99
Mastoid cavity	321	6	0

Data are number of temporal bones.

TABLE 3. TOPOGRAPHIC MEASUREMENTS (MEANS AND STANDARD DEVIATION) WITHIN TEMPORAL BONE SPECIMENS AT FOUR LEVELS

Topographic Sites	*Level I*	*Level II*	*Level III*	*Level IV*
FN-PSC or FN-LSC (A)	2.33 ± 0.57 (A1)	2.86 ± 0.54 (A2)	2.98 ± 0.41 (A3)	3.78 ± 1.16 (A4)
FN-ChT (B)	3.04 ± 0.43 (B1)	2.68 ± 0.34 (B2)	2.33 ± 0.34 (B3)	1.98 ± 0.39 (B4)
ST width (W)	1.06 ± 0.35 (W1)	1.49 ± 0.39 (W2)	1.37 ± 0.60 (W3)	1.16 ± 0.51 (W4)
ST depth (D)	1.08 ± 0.85 (D1)	2.06 ± 0.60 (D2)	1.63 ± 0.76 (D3)	1.32 ± 0.81 (D4)
Thickness of bone posterior to ST		3.19 ± 0.73 (E2)		
Thickness of bone within FR		1.58 ± 0.72 (F2)		

Data are mean ± SD in millimeters. Abbreviations as in Table 1.

lar temporal bones. Specimens that contained pneumatized compact bone posterior to the ST had a significantly larger interspace between the FN and the LSC at level I compared with specimens with acellular bone in this area. Also, the W and D of the ST at levels I through III were significantly larger in specimens containing pneumatized bone between the ST and the mastoid. The ST had an increased depth of 1 mm in those specimens containing pneumatized compact bone. Conversely, the thickness of the compact bone separating the ST from the mastoid air cells at level II was significantly greater in specimens in which this bone was acellular compared with those in which it was pneumatized (Table 4). Temporal bones in which the posterior wall of the ST is acellular have an average of 1.85 mm more bone between the ST and the mastoid cavity, compared to bones in which the posterior wall is pneumatized.

The size variation of the FN-ChT interspace, the size of the ST, and the thickness of the bone plate between the FR and the mastoid were assessed for systematic variation as a function of pneumatization of the bone contained within the FR (Table 5). At level II, between the stapedial tendon and the round window, the thickness of the compact bone within the FR was significantly greater in specimens containing acellular bone compared with pneumatized bone ($p < .0001$). The acellular specimens average an increased bone thickness of 1.36 mm compared to pneumatized FR cases. Also, in specimens containing pneumatized compact bone between the FR and the mastoid, the FN-ChT interspace at the round window (level III) was significantly greater compared with acellular bones ($p < .0431$). Pneumatization of the FN-ChT interspace had no effect on the degree of posterior extension or depth of the ST.

DISCUSSION

The ST has long been recognized as a challenging area in otologic surgery. It comprises a region of the posterior mesotympanum that is refractory to direct visualization. Removal of disease from this area often is limited to blind probing with blunt excavators in an attempt to remove any hidden disease. Since the ST is commonly invaded by cholesteatoma and/or granulation tissue,[5] addressing this area can be critical to the successful eradication of disease. Unsatisfactory visualization of the ST has led to the consideration of alternative approaches to this area. The FR approach, as suggested by Jansen,[6] provides access to some of the problem areas in the posterior tympanum. However, complete access to an unusually large ST is not possible from this approach (Fig 6). More radical procedures, such as the canal wall–down

TABLE 4. EFFECT OF PNEUMATIZATION OF BONE POSTERIOR TO SINUS TYMPANI ON TOPOGRAPHIC MEASURES

Site	*Level*	*Sclerotic (mm; mean ± SD)*	*Pneumatized (mm; mean ± SD)*	*ANOVA p*
FN-LSC	I	2.23 ± 0.07	2.65 ± 0.13	.0438
FN-PSC	II	2.80 ± 0.07	3.03 ± 0.13	.1408
FN-PSC	III	2.94 ± 0.05	3.11 ± 0.10	.8424
FN-PSC	IV	3.66 ± 0.14	4.15 ± 0.26	.1065
ST width	I	0.99 ± 0.04	1.29 ± 0.08	.0154
ST width	II	1.42 ± 0.05	1.73 ± 0.08	.5317
ST width	III	1.30 ± 0.07	1.59 ± 0.14	.9494
ST width	IV	1.09 ± 0.06	1.41 ± 0.11	.9032
ST depth	I	0.87 ± 0.11	1.75 ± 0.19	.3147
ST depth	II	1.79 ± 0.08	2.95 ± 0.14	.0027
ST depth	III	1.41 ± 0.09	2.34 ± 0.17	.0451
ST depth	IV	1.16 ± 0.10	1.84 ± 0.19	.1464
ST thickness	II	3.62 ± 0.09	1.77 ± 0.16	.0001

Abbreviations as in Table 1.

TABLE 5. EFFECT OF PNEUMATIZATION OF FACIAL RECESS ON TOPOGRAPHIC MEASURES

Site	*Level*	*Sclerotic FR (mm; mean ± SD)*	*Pneumatized FR (mm; mean ± SD)*	*ANOVA p*
FN-ChT	I	2.92 ± 0.08	3.09 ± 0.06	.4994
FN-ChT	II	2.53 ± 0.07	2.75 ± 0.05	.5793
FN-ChT	III	2.17 ± 0.06	2.40 ± 0.04	.0431
FN-ChT	IV	1.81 ± 0.08	2.05 ± 0.05	.2109
ST depth	II	1.82 ± 0.12	2.16 ± 0.08	.1904
ST depth	III	1.42 ± 0.15	1.72 ± 0.10	.3806
FR thickness	II	2.53 ± 0.14	1.17 ± 0.09	.0001

Abbreviations as in Table 1.

technique with removal of the posterior canal wall and ChT sacrifice, may still not adequately expose the ST.[7] The concept of endoscopic ear surgery has been introduced in recent years in an attempt to visualize hidden zones of the middle ear and the mastoid.[8] The ST is the most often mentioned inconspicuous region for which this technique may have a place. However, while endoscopes may improve visualization of this area, they do not solve the problem of removing the disease.

Messerotti et al[9] investigated the retrotympanum area in 25 fresh human temporal bones. The recess that was found to be present most consistently in the retrotympanum was the ST. The ponticulus, considered the superior boundary of the ST, was observed in only 50% of cases. The subiculum, known as the inferior margin of the ST, was observed in 72% of the cases. They found the depth of the ST to range from 1.5 to 6.5 mm, and the width of the ST ranging from 1 to 4.5 mm.[9] Similar findings were reported by Saito et al.[10] In the present series of 327 temporal bones the depth of the ST ranged from 0.2 to 9.9 mm (average 2.06 mm), and the width was 0.49 to 3.87 mm (average 1.49 mm). The distance between the FN and PSC at the level between the stapedial tendon and the round window averaged 2.86 mm. These findings are in close accordance with the literature for the ST dimensions.[9,10]

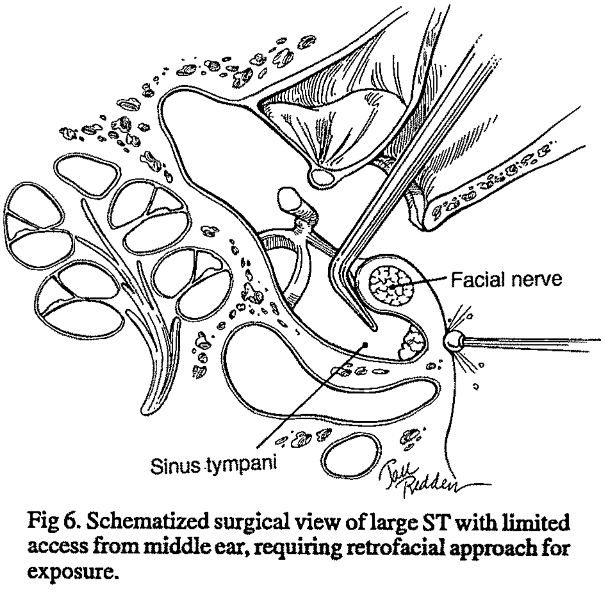

Fig 6. Schematized surgical view of large ST with limited access from middle ear, requiring retrofacial approach for exposure.

Reports in the literature demonstrate that the inner and middle ear structures do not alter their dimensions or anatomic relationships with aging.[10-13] Young and Nadol[14] did not find any significant difference in the dimensions of the extended FR with age, sex, or pneumatization. In the present study, the average maximum width of the ST was 1.56 mm in males and 1.40 mm in females and the average maximum depth of the ST was 2.15 mm in males and 1.95 mm in females. Consistent with previous reports, these differences were not found to be significant.

This study did find a consistent effect of pneumatization on ST topography. Comparisons were made between temporal bones in which the posterior wall of the ST was pneumatized and those in which it was acellular. We found that at level II the average FN-PSC interspace was 2.80 mm in acellular cases, the average D of the ST was 1.79 mm, and the compact bone between the ST and mastoid air cells averaged 3.62 mm. In bones containing a pneumatized posterior wall of the ST, the FN-PSC interspace averaged 3.03 mm, the average D of the ST was 2.95 mm, and the average W of the compact bone between the ST and mastoid air cells was 1.77 mm. While the differences in the measurements of bone plate thickness posterior to the ST and ST D were significant, there was no significant difference in FN-PSC interspace size between cases in which the posterior wall of the ST was pneumatized or acellular, except at the level of the stapedial tendon. According to Proctor and Nager's[15] detailed study of FN anatomy, the distance separating the FN from the summit of the angle formed by the horizontal and posterior semicircular canals is at most 3 to 4 mm. Medially, the second turn of the FN is separated from the ampulla of the PSC by 4 to 5 mm of compact bone in pneumatized temporal bones and by only 2 mm of compact bone in sclerotic temporal bones.[15] There were no sclerotic temporal bones examined in the current study.

It is well known that the ST displays significant variability in its size and posterior extent. This variability was also demonstrated in the present study. The clinical relevance of these topographic variations is that one can predict the potential risk of inadequately treating an unusually large ST in those specimens containing a pneumatized posterior wall. This occurred in nearly 25% of the specimens examined in this study. These specimens also had a narrower plate of bone interposed between the ST and the mastoid air cells than the acellular specimens. Potentially, then, the cases that would most benefit from a posterior approach to the ST between the FN and the labyrinth would have favorable anatomy for this approach. The feasibility of a retrofacial approach to the ST hinges on access between the FN and the labyrinthine structures. Experience with the FR approach to the mesotympanum has shown that this is surgically possible in most temporal bones. The FR approach provides access to the oval window region and the posterior mesotympanum. Exposure of the ST is limited by the posterior canal wall and the descending portion of the FN.[16] In this study the size of the FN-ChT interspace ranges from 1.98 to 3.04 mm. The size of the FN-PSC interspace was equal to or greater than the size of the level II FN-ChT interspace, the area of the FR approach, in 64.5% of specimens. In other words, at all levels below the stapedial tendon the FN-PSC interspace was greater than the FN-ChT interspace in nearly two thirds of the temporal bones examined. This suggests that an approach to the medial mesotympanum via the ST is possible.

High-resolution computed tomography (CT) has been useful in obtaining additional evidence of ST invasion by cholesteatoma. Bone destruction in the posterosuperior part of the tympanic annulus, the lateral wall of the FR, and the pyramidal eminence is an important finding for suspected invasion of the ST area by cholesteatoma.[17] In addition, preoperative CT scanning can provide important information regarding the D of the ST and whether the bone surrounding the FN in the descending portion is pneumatized or acellular.

The retrofacial air cell tract and its anatomic importance had been emphasized by Benecke et al.[18] It is possible to identify the FN, endolymphatic sac, and jugular bulb without injury to the inner ear, because of the constant presence of this cell tract. This cell group is located posterior and medial to the FN. Superiorly, they are in contact with the deep subantral cells around the LSC and PSC, and inferiorly, they extend over the sigmoid sinus and jugular bulb.[19] We have also found that a well-pneumatized retrofacial air cell tract was present in almost all of the temporal bones studied. The retrofacial air cell tract provides a consistent and surgically relatively safe starting point for dissection of the ST. The tract can be followed superiorly and laterally to the PSC as an entry point to the posterior ST (Fig 6).

The posterior approach to the ST should be feasible for all levels inferior to the stapedial tendon. A preoperative CT scan showing a well-developed ST with pneumatization of the bone between the ST and the mastoid air cells suggests anatomy favorable to this approach. Forward positioning of the sigmoid sinus, a superior location of the jugular bulb, lateral positioning of the PSC, a medially shifted FN, or a poorly developed ST all potentially complicate this posterior approach to the ST.

CONCLUSIONS

This study demonstrates that the distance between the labyrinth and the FN is greater than the distance between the FN and ChT in all levels, except in the region of the stapedial tendon. Therefore, a retrofacial approach to the ST should be feasible at all levels except that of the stapedial tendon. Favorability is increased if this area is pneumatized. While preoperative CT scanning can be helpful in selecting favorable cases, intraoperative findings will dictate the necessity for the retrofacial ST approach. If CT scanning shows a shallow ST or lack of posterior expansion of the ST, a highly placed jugular bulb close to the bony labyrinth, or a laterally positioned PSC, the approach is contraindicated. Likewise, intraoperative findings of an unfavorable position of the sigmoid sinus or jugular bulb or FN variations contraindicate this approach.

The ST remains a difficult site in the middle ear that can contain disease and that can be challenging to address. The usual approach to this area is to perform blind probing in an attempt to eradicate any retained epithelial remnants or granulation tissue. We have studied over 300 temporal bones to further describe the anatomic dimensions of the ST. We conclude that in up to 65% of cases the ST can be approached from the mastoid in a retrograde fashion. This is essentially a surgical extension of the consistently present retrofacial air cell tract superiorly between the FN and the labyrinth. We recommend high-resolution CT of the temporal bone to assess the pneumatization and depth of the ST and surrounding bone prior to attempting this new approach, because the surgical dimensions are so small and the tolerance for error is minuscule.

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Ann Otol Rhinol Laryngol 105:1996

PNEUMATIZATION OF THE TYMPANIC BULLA AFTER BLOCKAGE OF THE VENTILATION ROUTE THROUGH THE EUSTACHIAN TUBE IN THE PIG

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To examine whether gas exchange occurs in the middle ear air cell system independent of the eustachian tube (ET), we occluded the middle ear clefts of piglets, whose tympanic bullae closely resemble the human mastoid air cell system, and investigated subsequent changes in the air cell system. We anticipated that pneumatization and development of the air cell system would continue if gas were exchanged through the mucosa lining the air cell system. If, on the other hand, mastoid air cells depend on the ET, mastoid development would be impaired or arrested. In noninflamed ears, pneumatization was maintained, and development of the air cell system continued after the middle ear cleft had been occluded with an acrylate adhesive and the bullar air cell system was thus excluded from any communication with the ET. These results indicate that the normal middle ear air cell system has the capacity to perform gas exchange independently of the ET, with gas exchange appearing to occur through the submucosal capillary network.

KEY WORDS — experimental study, histomorphometry, mastoid air cell system, middle ear physiology, middle ear ventilation, pig.

INTRODUCTION

It has generally been considered that gas in the middle ear cavity is continuously absorbed into the surrounding tissue, and fresh air is supplied to the middle ear cleft and the mastoid air cell system through the eustachian tube (ET) to compensate for the absorbed gas.[1-3] However, some reports indicate that gas is generated or exchanged through the middle ear mucosa,[4-7] and gas exchange in the mastoid air cell system is thus regarded as important in middle ear ventilation and pressure regulation.[8] To examine whether gas exchange occurs in the middle ear air cell system, we occluded the middle ear clefts of piglets. The tympanic bullae of pigs closely resemble the human mastoid air cell system, and blocking the ventilation route from the ET to the air cell system would tell us if, in fact, mastoid ventilation is dependent on the ET. We investigated subsequent changes in the air cell system. We felt that if pneumatization and development of the air cell system continued with the ET permanently obstructed, gas was being exchanged and supplied through the mucosa lining the bullar air cell system, because resupply through the ET was precluded by the obstruction.

MATERIALS AND METHODS

Ten piglets (sire, Landrace-Hampshire crossbred; dam, Duroc) were used for this experiment. Four animals (8 ears) served as the untreated normal controls, and 6 animals (12 ears) were the experimental group. In the 4 animals of the normal control group, bilateral tympanic bullae of each animal were removed 1, 3, 4, and 6 months, respectively, after birth. The 6 animals of the experimental group were subdivided into 3 groups of 2 animals each and treated as follows.

Group 1. In group 1, both tympanic membranes were perforated, and pure glycerin was injected into the middle ear at age 1 week. Jellied instant adhesive (α-cyanoacrylate monomer, Aron Alpha A, Sankyo) was injected into the middle ear at age 1 month. The mastoid bullae were removed 3 months after injection of the acrylate (age 4 months).

Group 2. In group 2, the bilateral tympanic membranes were perforated, and adhesive alone was injected into the middle ears at age 1 month. The bullae were removed at age 4 months.

Group 3. In group 3, only acrylate was injected into the middle ear at 3 months. The bullae were not removed until 6 months after birth and 3 months after injection of the acrylate.

The reason for instilling glycerin first at 1 week and acrylate 3 weeks later in the middle ear clefts of piglets in group 1 was to determine if there would be a difference between ears with an obstructed and

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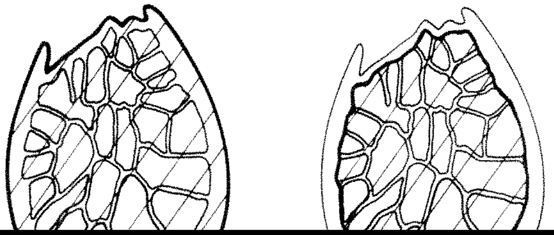

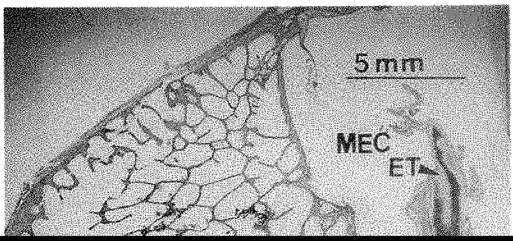

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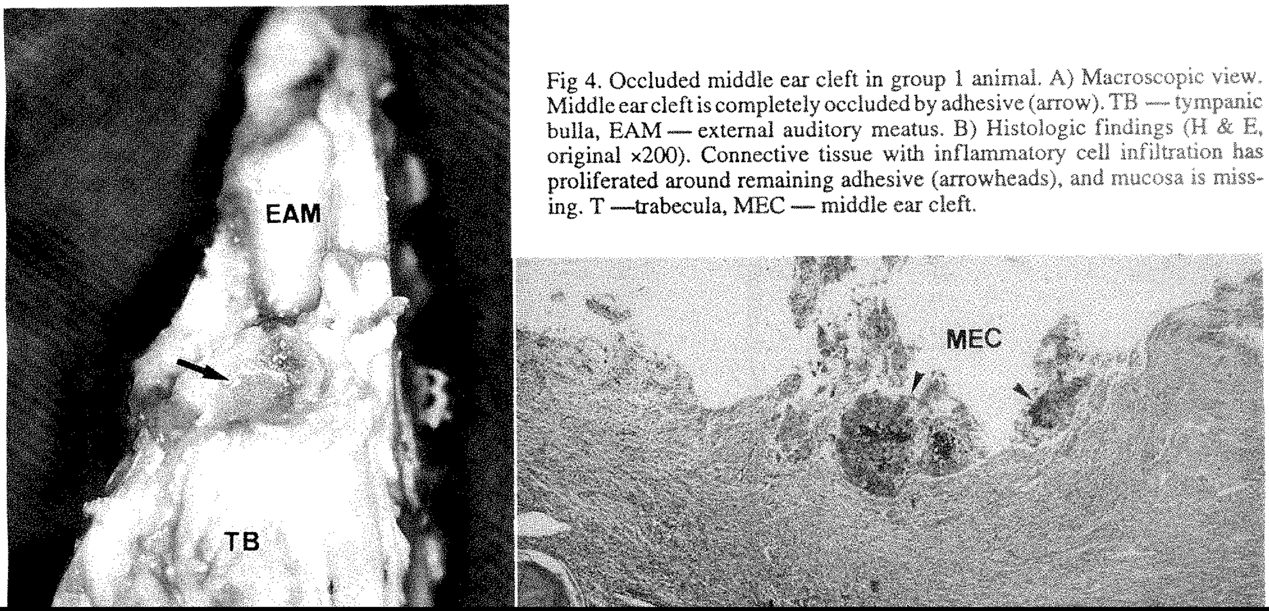

Fig 4. Occluded middle ear cleft in group 1 animal. A) Macroscopic view. Middle ear cleft is completely occluded by adhesive (arrow). TB — tympanic bulla, EAM — external auditory meatus. B) Histologic findings (H & E, original ×200). Connective tissue with inflammatory cell infiltration has proliferated around remaining adhesive (arrowheads), and mucosa is missing. T —trabecula, MEC — middle ear cleft.

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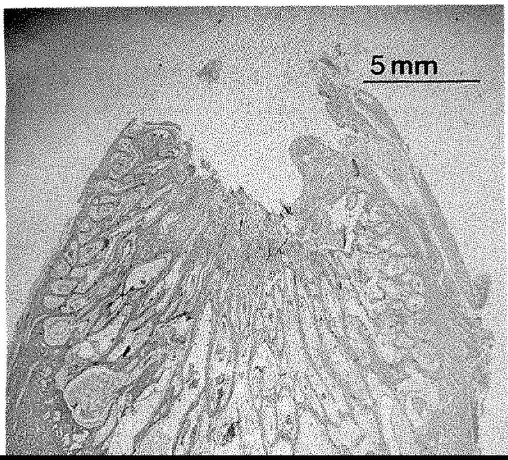
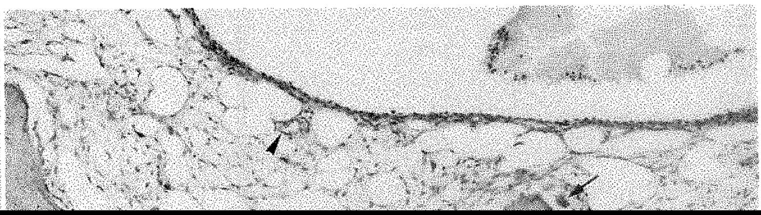


Fig 6. Tympanic bulla from group 2 animal. A) Although development of air cell system is slightly inhibited, degree of inhibition of pneumatization is less than that in group 1 (H & E). Pneumatized cells are observed in peripheral sites of air cell system. B) Inner surface of cortex (H & E, original ×100). Osteoclast numbers have diminished compared with those in control group, but osteoclasts still remain (arrows). Capillaries (arrowhead) are observed in subepithelial connective tissue.



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tained in the peripheral cells even after the ventilation route to the middle ear cleft had been blocked. Histomorphometric analysis (see Table) shows that the area of the air cell systems in group 2 ears (removed at 4 months) was greater than that of the control ears removed 1 month after birth, and the area of the systems in group 3 (removed at 6 months) was greater than that of control ears removed 3 months after birth. Development of the air cell system in groups 2 and 3 progressed satisfactorily, although not to the same extent as seen in the controls. We therefore concluded that osteoclastic activity continues after middle ear occlusion. Osteoclast numbers were diminished by the inflammation evoked by the adhesive, and there were accompanying decreases in pneumatization, which were less than that in the controls.

These findings suggest that development of the middle ear air cell system was markedly suppressed by prolonged inflammation, secondary to glycerin, and that pneumatization of early-formed bullar cells could not occur because of stagnation of effusion secondary to the inflammation. In noninflamed ears (groups 2 and 3), pneumatization and development of the air cell system did occur, despite obstruction of the ventilation route to the air cells in the middle ear.

The phenomenon of pneumatization continuing in an air cell system with no connection to the ET or the middle ear cleft cannot be explained by the classic concept of absorption and resupply of air by the ET to the air cell system. It is necessary to consider another ventilation route apart from the ET and the middle ear cleft. Okubo and Watanabe[8] consider that gas is generated in the mastoid air cell system and postulate the presence of a gas exchange route through the submucosal capillary network in the mucosa lining the air cell system. We noted a capillary network in the submucosa of the middle ear air cell system in the pig, which persists even after middle ear occlusion. We therefore consider that under these conditions, pneumatization can be maintained and development of the air cell system may progress by gas exchange passing through the capillary network. The results of our experiment indicate that the air cell system itself has the capacity to allow gas exchange.

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MORPHOLOGIC CHANGES IN CONTRALATERAL SUPERIOR VESTIBULO-OCULAR NEURONS FOLLOWING LABYRINTHECTOMY IN THE CAT

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Ultrastructural changes were measured in 22 contralateral superior vestibulo-ocular neurons (SVONs) from four cats painlessly sacrificed at 8 weeks and 25 contralateral SVONs from four cats sacrificed at 1 year following unilateral labyrinthectomy. The SVONs at 8 weeks showed a 43% decrease in somal size, a 34% loss in rough endoplasmic reticulum (RER), a 48% loss of ribosomes, and a 47% decrease in the number of synaptic profiles (SPs) contacting the soma. At 1 year the SVONs had a 31% decrease in size, a 43% decrease in RER, a 50% loss of ribosomes, and a 71% decrease in SPs. Synaptic vesicles (SVs) in both groups of SVONs showed no change in size or shape compared to control SVs. These findings resemble the contralateral SVON changes that occur following excision of the vestibular ganglion.

KEY WORDS — labyrinthectomy, ultrastructure, vestibulo-ocular neurons.

INTRODUCTION

Knowledge of the morphologic changes in brain stem neurons following vestibular ablation is important in our understanding of the extent and degree of change in the vestibular nuclei following peripheral ablation. Such information may guide the direction and interpretation of histochemical and molecular genetic studies that will be important in understanding the plastic changes occurring during compensation.[1-3]

Over the past 10 years we have described quantitatively the morphologic changes that occur in a population of second-order neurons in the superior vestibular nucleus (superior vestibulo-ocular neurons; SVONs) following two forms of peripheral ablation: vestibular ganglionectomy and labyrinthectomy.[4-8] These reports have documented, in addition to the expected transneuronal atrophy and loss of synaptic profiles (SPs) on the ipsilateral SVON, that extensive transneuronal changes ensue in the contralateral SVON following ganglionectomy.[6,7] Recently, we reported transneuronal atrophy and progressive loss of SPs on SVONs as well as first-order vestibular neurons ipsilaterally following labyrinthectomy.[8] In the present report, the changes in the contralateral SVON 8 weeks and 1 year following labyrinthectomy are described. Transneuronal SVON changes similar to those seen after ganglionectomy were found.

MATERIALS AND METHODS

Horseradish peroxidase (HRP)–labeled contralateral SVONs were obtained from four cats painlessly sacrificed at 8 weeks, and four cats at 1 year, following labyrinthectomy. Data on the ipsilateral SVONs from these animals were described in a previous report.[8]

The techniques for labyrinthectomy, labeling of SVONs with HRP, and animal fixation have been previously described.[4,8] These reports also contain the methods for harvesting, staining, and preparation of ultrathin sections of labeled cells for transmission electron microscopy. The quantitative morphometric techniques used to measure changes in the SVONs are the same as those used in earlier studies.[4]

RESULTS

Twenty-two contralateral SVONs from four 8-week postlabyrinthectomy cats and 25 contralateral SVONs from four 1-year postlabyrinthectomy cats were studied. The Table summarizes the changes in somal size, and volume fractions of nucleus and cell organelles (mitochondria, Golgi apparatus, rough endoplasmic reticulum [RER], ribosomes). The average numbers of SPs contacting the somal membrane were also measured. There was a 43% decrease

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MORPHOMETRIC COMPARISON OF VESTIBULO-OCULAR NEURONS AFTER LABYRINTHECTOMY

	Control Neurons	Contralateral Neurons	
		8 wk	*1 y*
Mitochondria (%)	12.4 ± 3	12.7 ± 3	12.2 ± 2
Nucleus (%)	17.5 ± 4	24.0 ± 6	21.9 ± 7
Rough endoplasmic reticulum (%)	6.5 ± 3	4.3 ± 2	3.7 ± 2
Golgi apparatus (%)	4.6 ± 1	4.2 ± 1	4.5 ± 1
Polyribosomes (%)	28.4 ± 7	14.9 ± 2	14.2 ± 3
Area cell (μm^2)	928 ± 293	525 ± 143	644 ± 252
No. of synaptic profiles per grid	16 ± 5	8.5 ± 4	4.6 ± 3
No. of cells in group	26	22	25

Data are mean ± SD, except for last entry.

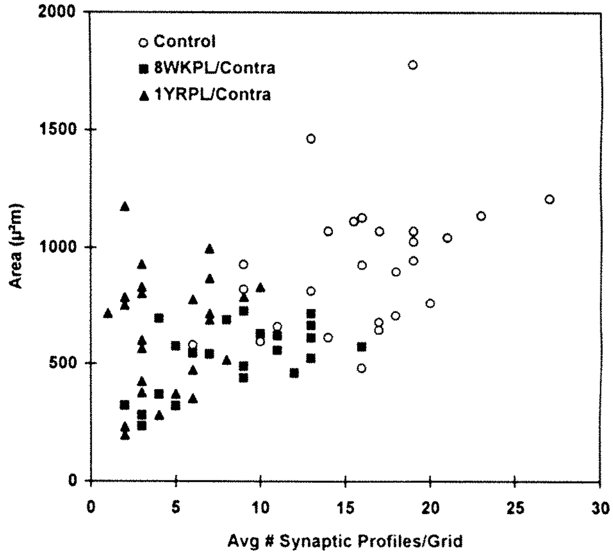

Fig 1. Scatter plot of superior vestibulo-ocular neurons shows further degeneration of synaptic profile at 1 year compared to 8 weeks postlabyrinthectomy (PL).

in somal size at the 8-week survival period and a 31% decrease in size at 1 year following labyrinthectomy. The increase in volume fraction of the nucleus was only relative because of decreased somal size, which is used as the reference volume. Nuclear membrane infolding characteristic of transneuronal atrophy was observed in several cells. In others the nuclear membrane was of normal contour or showed slight undulations. There were no significant changes in the volume fractions of mitochondria or the Golgi apparatus compared to control cells. However, significant decreases were observed in the volume fractions of RER and ribosomes of experimental cells. At 8 weeks postlabyrinthectomy the RER was decreased 34%, while at 1 year the decrease was 43%. Polyribosomes were decreased 48% and 50% at the 8-week and 1-year survival periods, respectively. Transneuronal atrophy remained constant, but there was a progressive loss of SPs from 8 weeks to 1 year postlabyrinthectomy (47% at 8 weeks and 71% at 1 year; Fig 1). Ongoing degeneration of SPs was observed in the 1-year SVONs (Fig 2).

The size and shape of digitized synaptic vesicles (SVs) measured in the SPs contacting the SVONs are summarized in Fig 3. There were 10,199 SVs digitized in the 8-week SVONs, and 6,793 SVs in 1-year SVONs. There was no significant size or shape change at either time period compared to control SVs (98,000).

Synaptic membrane specializations were classified as symmetric or asymmetric for the SVONs in both survival groups. In the 8-week SVONs there were 44 synapses classified as asymmetric and 136 symmetric, which represents a ratio of 0.32. At 1 year postlabyrinthectomy, 103 asymmetric and 91 sym-

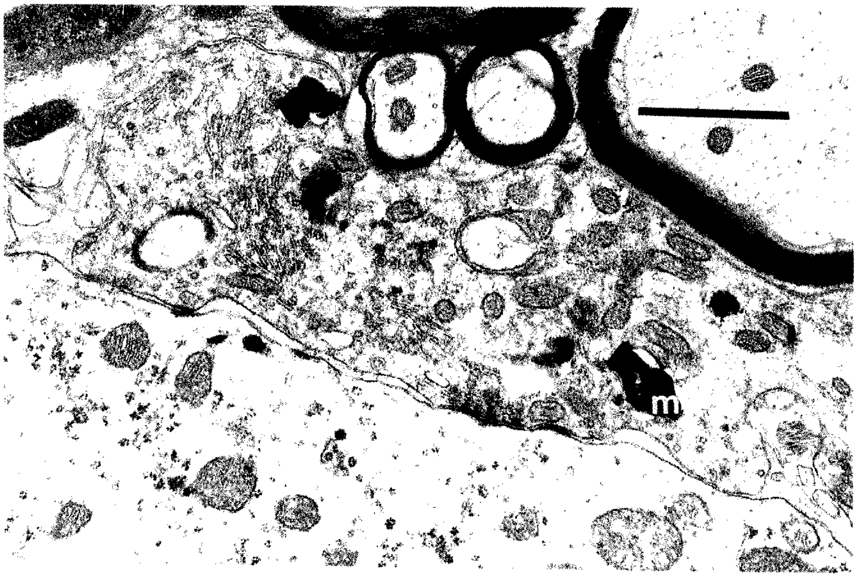

Fig 2. Electron micrograph of degenerating mitochondria (m) in synaptic profile on contralateral SVON 1 year following labyrinthectomy (original ×25,250). Bar — 1 μm.

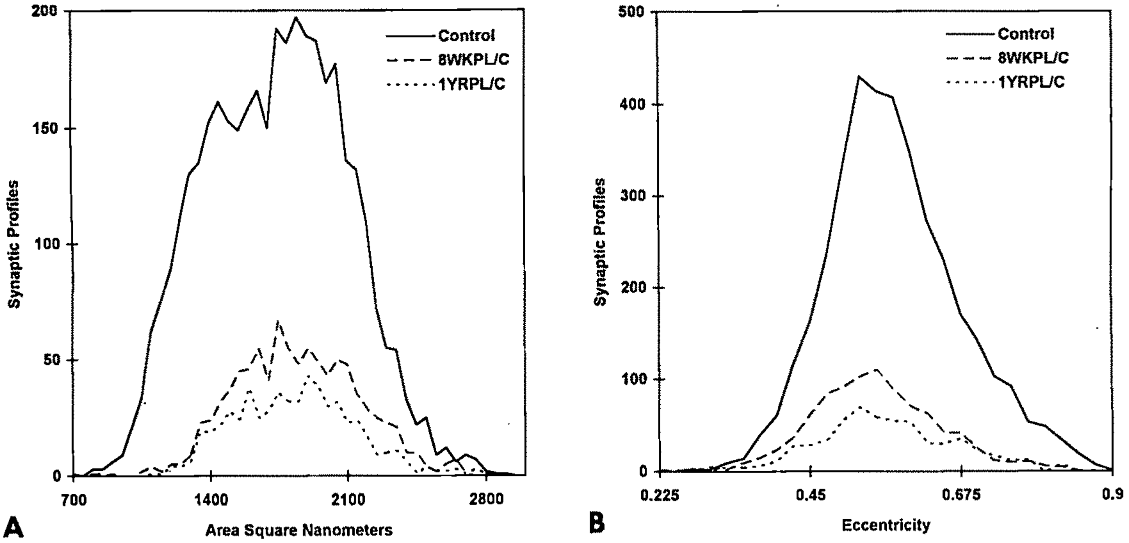

Fig 3. Curves of contralateral (C) synaptic vesicle A) size and B) shape at 8 weeks and 1 year postlabyrinthectomy (PL). In B, roundness is represented at left and flatness to right.

metric synapses were classified, representing a ratio of 1.13.

Excitatory synapses containing Taxi bodies[9] were seen in both groups of SVONs. Seven such synaptic arrangements were seen in the 1-year contralateral SVONs, while five were observed in the 8-week SVONs. Two synapses with Taxi bodies were found in a comparable number of ipsilateral SVONs at 1 year postlabyrinthectomy.[8]

DISCUSSION

While contralateral changes were expected in view of our previous data demonstrating contralateral SVON changes following vestibular ganglionectomy,[6,7] the degree was surprisingly high. Eight weeks after labyrinthectomy these changes were nearly double those found on ipsilateral SVONs in the same animals (25% decrease in SPs and 20% decrease in somal size). After 1 year the contralateral SVONs showed a greater loss of SPs (71%) and size (31%) compared to ipsilateral SVONs (SPs 60%; size 21%). The magnitude of change is difficult to explain. In other studies that have described transneuronal changes following interruption of an afferent pathway, a 20% decrease appears to be the norm.[10] It seems unlikely that the loss of a single afferent input (commissural) can explain such drastic contralateral changes.

Since no primary vestibular nerve fibers cross the midline, an expected contralateral effect would be mediated transneuronally over crossing pathways such as the commissural or cerebellar pathways.[11-13] Prominent commissural projections connect the superior and medial vestibular nuclei.[14,15] These are primarily inhibitory and restricted to canal activity.[11] Cerebellar contralateral projections to the superior nucleus could originate from the caudal cerebellar vermis (nodulus, uvula), where primary vestibular neurons terminate,[16-20] or from the fastigial nucleus, which receives an indirect connection from the vestibular cerebellum.[21]

The vestibular nerve input to SVONs accounts for about 70% of the somal SPs, and denervation produces a 20% decrease in somal size.[4,5] Therefore, a transneuronal degeneration over crossing pathways should only account for about 30% of the SPs on SVONs and the expected decrease (20%) in somal size that follows cessation of a neural input. The magnitude of SP loss and cell atrophy at 8 weeks (47% and 43%, respectively) and at 1 year (71% and 31%, respectively) suggests a combined loss of the SPs from both sources as responsible for this magnitude of change.

We have established that the vestibular nerve SPs on SVONs contain larger SVs than the SPs of other input systems (commissural, cerebellar).[5-7] Following acute removal of vestibular nerve SPs (ganglionectomy), the average size of SVs in intact SPs was 1,400 nm^2. Since the average SV size in controls is 1,700 nm^2, the loss of larger SVs is caused by vestibular nerve SP degeneration. If the SP loss on contralateral SVONs resulted from degenerating commissural (or cerebellar) pathways, the average SV size should be larger than the average of control SVs. Instead, there was no significant SV size change compared to control.

We propose that the substantial changes in contralateral SVONs following ablation result from crossing systems shutting down, as well as a compensatory loss of SPs belonging to the contralateral vestibular nerve. This latter phenomenon was noted at 8 weeks[8] and accounts for the progressive degeneration of contralateral nerve SPs found at 1 year postablation. This matches the number of SPs found on ipsilateral SVONs. We referred to this event as a "pruning" of the cluster of SPs supplied by single vestibular axons.[7] This explains the reduced number of SPs contacting the soma while the number of vestibular ganglion cells remains normal.[7,8]

The ratios of asymmetric (excitatory) to symmetric (inhibitory) synapses in the contralateral SVONs were comparable to those found in the contralateral SVONs after ganglionectomy. Eight weeks following labyrinthectomy, the ratio was 0.32, while after ganglionectomy it was 0.25. Compared to control SVONs (0.14), these ratios represent a relative increase in the number of excitatory synapses. These ratios were comparable to the ratio found ipsilaterally on SVONs at 8 weeks and 1 year following ganglionectomy (0.40 and 0.37) and labyrinthectomy (0.31 and 0.24). However, a marked increase in this ratio was seen at the 1-year period following both ganglionectomy (1.47) and labyrinthectomy (1.13). It is possible that the increased number of asymmetric synapses represents a mechanism by which the contralateral vestibular nerve can exert an effective excitatory input to the SVONs over a normal number of axons acting through a reduced number of SPs. Although the data on observed Taxi bodies are insufficient to support a conclusion, they do support a concept of increased excitatory activity in the contralateral SPs. Insufficient information is available at this time to explain this observation.

These studies provide a quantitative measure of the morphologic changes that occur in an important second-order neuron following these two types of ablation procedures. Similar changes in other brain stem vestibular neurons are likely. Immunohistochemical and molecular genetic approaches are needed to provide insight into functional changes occurring in these neurons. The interpretation of such studies will be greatly facilitated with the knowledge of the morphologic changes that occur during the compensation process.

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STUDY ON ADHESION FACTORS IN LYMPHOCYTE MIGRATION TO THE MIDDLE EAR MUCOSA

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We investigated influences of adhesion factors on the migration of antigen-specific IgA-forming cells (ASAFCs) to the middle ear mucosa by means of an in vitro lymphocyte binding assay. Peyer's patch (PP) lymphocytes from guinea pigs with mucosal immunization, which are rich in ASAFCs, more frequently bound with the inflamed middle ear mucosa than those of PP and spleen cells from animals with systemic immunization, in which antigen-specific IgG-forming cells (ASGFCs) were induced ($p > .001$). The bindings were not affected by antigenic and nonantigenic stimuli to the middle ear mucosa for producing otitis media. On human middle ear mucosa from 10 patients with acute mastoiditis and chronic otitis media, endothelial cells of newly grown vessels were stained strongly with intercellular adhesion molecule (ICAM)–1, and weakly with vascular cell adhesion molecule (VCAM)–1, platelet endothelial cell adhesion molecule (PECAM), and endothelial leukocyte adhesion molecule (ELAM)–1. Many lymphocytes bound mainly to these endothelial cells, and a few cells were observed bound to the basal portion of epithelial cells. The binding of lymphocytes was significantly, but not completely, inhibited by anti–ICAM-1 antibody ($p < .001$). These findings suggest that PP lymphocytes with activated mucosal immunity more frequently migrate to the inflamed middle ear mucosa, and that those migrations, after extravasation, may be regulated by the interaction between various binding factors and their receptors on lymphocytes, which is different from that of adhesion molecules and their ligands in the extravasation.

KEY WORDS — adhesion molecules, antigen-specific IgA-forming cells, lymphocyte migration, middle ear mucosa.

INTRODUCTION

After enhancement of the mucosal immunity, antigen-specific IgA-forming cells (ASAFCs) appear in the inflamed mucosa of the middle ear cavity and eustachian tube.[1] These IgA-forming cells are derived from mucosa-associated lymphoid tissues (MALTs) such as gut-associated lymphoid tissue (GALT) and bronchus-associated lymphoid tissue (BALT).[2] Those precursors of IgA plasma cells are generated in germinal centers of those lymphoid tissues in which heavy chain class switching to α-chain occurs in the cells.[3] The precursors enter into the blood circulation via the draining lymphatics and thoracic lymphatic duct and maturate to IgA-forming cells in effector sites after systemic circulation. They are thought to migrate into various local sites of the mucosae and exocrine glands via the systemic circulation. However, this migration mechanism and the antigenic effects of the migration are still unclear. Several studies[4-8] have demonstrated that various adhesion molecules, to which lymphocytes are bound, are present on the surface of vascular endothelial cells. This suggests that the interaction between those adhesion molecules on the endothelial cells and their ligands on lymphocytes may play an important role in lymphocyte migration to the local sites. The binding of lymphocytes to endothelial cells lining the postcapillary high endothelial venules (HEVs) is thought to be the first step in lymphocyte migration. Through this binding, those cells may extravasate from the bloodstream into local sites. These processes can account for the extravasation, but not for the cells' local lodging and retention at local sites.

Recently, it has been reported that a receptor-ligand interaction between lymphocytes and glandular tissues, which is different from a lymphocyte-HEV interaction, mediates the localization of IgA lymphocytes in the exocrine glands.[9,10] Our previous study also suggested the presence of organ-specific binding factors to which B lymphocytes bind specifically, in the inflamed middle ear mucosa, as well as in the salivary gland.[11] However, it is not clear whether or not those binding factors and adhesion molecules are present on the middle ear mucosa and participate in lymphocyte migration. In order to clarify the mechanism of migration of ASAFCs to the middle ear mucosa, we investigated the influences of antigenic and nonantigenic stimuli to the middle ear mucosa on lymphocyte migration and the distribution of various adhesion molecules on the middle ear mucosa.

MATERIALS AND METHODS

Animals. Healthy male Hartley guinea pigs (age, 4 to 6 weeks old; body weight, 300 to 400 g) were used in this experiment.

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Preparation of Antigen. Dinitrophenylated ovalbumin (DNP-OVA) was prepared as an antigen and conjugated with formalin-killed *Streptococcus mutans* strain 6715 as a particulate form of DNP-OVA antigen, as previously reported.[1]

Antisera. Rabbit antisera against guinea pig α- and γ-chains (ICN Immunobiologicals, Lisle, Ill) and goat antisera against rabbit IgG conjugated with horseradish peroxidase and alkaline phosphatase (Cappel, Novern, Pa) were purchased from commercial sources. Mouse monoclonal antibodies against human adhesion molecules, such as intercellular adhesion molecule (ICAM)–1, vascular cell adhesion molecule (VCAM)–1, platelet endothelial adhesion molecule (PECAM), and endothelial leukocyte adhesion molecule (ELAM)–1, were also obtained from commercial sources (British Biotechnology, Oxford, England).

Induction of Antigen-Specific Antibody-Forming Cells of IgA and IgG Classes. Forty guinea pigs were immunized with DNP-OVA for the induction of antigen-specific antibody-forming cells of IgA and IgG classes (ASAFCs and ASGFCs) according to the immunization strategy previously described.[1] Briefly, 1 week after systemic priming, an intraduodenal immunization with the particulate antigen of DNP-OVA was given to half of the 40 animals for inducing ASAFCs, and a systemic booster with DNP-OVA was given to the other half for ASGFCs. Two weeks after priming, saliva and sera were collected, and antibody titers of IgA and IgG classes against DNP-OVA were determined in those samples by the indirect technique of enzyme-linked immunosorbent assay (ELISA) previously described.[1] In 14 of 20 animals with mucosal immunization, IgA antibody titers in saliva were elevated to more than 2^4, and serum IgG antibody titers reached more than 2^6 in 16 of 20 animals with the systemic booster. In this study, those animals with high titers of IgA and IgG antibodies were used for activated mucosal immunity (AMI) and activated systemic immunity (ASI). From those animals with AMI and ASI, Peyer's patches (PPs) and spleens were obtained as sources of ASAFCs and ASGFCs, respectively. ASAFCs and ASGFCs in those lymphoid tissues were detected by the method of a solid phase ELISA plaque-forming assay, as previously described.[1] The ASAFCs were 78 ± 11 cells per 1×10^5 cells in PPs from animals with AMI, and ASGFCs were 129 ± 17 cells per 1×10^5 cells in spleen from animals with ASI. The ASAFCs were 56.5% of IgA-cell fraction in PPs from animals with AMI, and the ASGFCs were 62.3% of IgG-cell fraction in the spleen from animals with ASI.

Preparation of Lymphocytes. As sources of autologous lymphocytes, PPs and spleen were obtained from animals with AMI or ASI, and lymphocytes were separated by gradient centrifugation. T and B lymphocytes were fractionated by a nylon wool column,[12] and fractions rich in IgA and IgG cells were prepared by treatments with rabbit anti guinea pig IgG and IgA antibodies, according to the method of Bianco et al.[13] The vital staining of lymphocytes with tetramethyl rhodamine-5-isocyanate (TRITC) was performed according to the technique of Butcher et al.[14] Finally, the stained cells were resuspended at a concentration of 2 to 3 × 10^8 cells per milliliter in RPMI 1640 medium supplemented with 5% fetal calf serum.

Antigenic and Nonantigenic Stimulation of Middle Ear Mucosa. For antigenic stimulation, 100 μg of DNP-OVA in 100 μL of saline and, for nonantigenic stimulation, 1×10^8 *Pneumococci* biotype III suspended in 100 μL of saline were inoculated into the right tympanic cavity through the tympanic membrane for inducing antigenic and nonantigenic inflammation of the middle ear mucosa. Half of the animals in each group with AMI or ASI had an intratympanic inoculation with DNP-OVA. In the other half of the animals with AMI or ASI, pneumococcal otitis media was induced. The left tympanic cavity, as a control ear, was injected with 100 μL of saline. Two days after intratympanic inoculation, the middle ear mucosa and various mucosal tissues including the nose, pharynx, and trachea, plus the small intestine and salivary glands, were obtained for in vitro lymphocyte binding assay.

Binding Assay of Lymphocytes to Various Mucosae and Salivary Glands. Various tissue samples, such as middle ear, nasal, pharyngeal, tracheal, and small intestinal mucosae, plus salivary glands, were obtained from animals with AMI or ASI. The middle ear mucosae were obtained from the distal portion of the tympanic bulla, and nasal mucosae were taken from turbinates. Fresh tissue samples were immediately frozen in liquid nitrogen, and serial 10-μm-thick frozen sections were prepared. Autologous lymphocytes from PPs and spleen were applied to each tissue section for a lymphocyte binding assay. One hundred microliters of vital stained autologous lymphocyte suspension at a concentration of 2 to 3 × 10^8 cell per milliliter was added to each tissue section and incubated for 30 minutes at room temperature. After fixation in 1% glutaraldehyde solution, excessive lymphocytes on the section were washed off with 0.01 mol/L phosphate-buffered saline at pH 7.4, and tissue sections were stained by methyl green. Binding lymphocytes on 20 sections of various mucosae were observed under a 400× magnified microscope, and

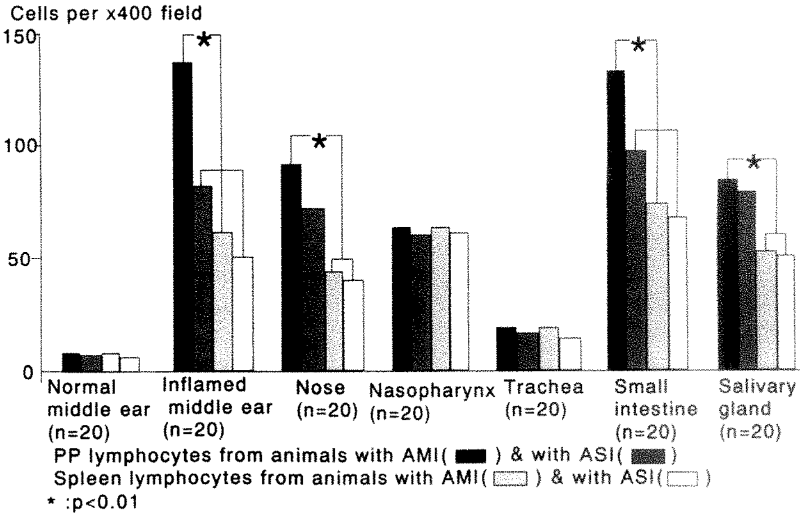

Fig 1. Mean numbers of lymphocytes binding to various mucosae and salivary glands. PP — Peyer's patch, AMI — activated mucosal immunity, ASI — activated systemic immunity.

binding lymphocytes per field 400× magnified were quantified by an image processor (Nexus 600). Various lymphocyte fractions, such as T cell, B cell, IgA, and IgG cell fractions, were applied to tissue sections, and binding lymphocytes of those fractions were quantitatively analyzed to evaluate the binding activity of each fraction.

Distribution of Various Adhesion Molecules in Human Middle Ear Mucosa. Human middle ear mucosae were obtained from five patients with chronic otitis media and from five patients with acute mastoiditis, and fresh mucosal tissues were taken from the antrum and epitympanum during surgery. Serial 10-μm-thick frozen sections of each sample were stained with various monoclonal antibodies against human adhesion molecules, such as ICAM-1, VCAM-1, PECAM, and ELAM-1. For immunohistochemical staining, the avidin-biotin complex technique was employed.[15]

Influences of Various Human Adhesion Molecules on Lymphocyte Binding to Human Middle Ear Mucosa. In order to investigate influences of various human adhesion molecules on lymphocyte migration to the middle ear mucosa, tissue sections of human middle ear mucosa were pretreated by monoclonal antibodies against various human adhesion molecules, such as ICAM-1, VCAM-1, PECAM, and ELAM-1. Tissue sections were incubated with each antiserum against adhesion molecules at 37°C for 30 minutes. After incubation, those sections were washed three times with phosphate-buffered saline. Autologous lymphocytes were prepared from peripheral blood from the same patient and stained with TRITC according to the technique of Butcher et al.[14] Vital stained autologous lymphocytes were applied to serial sections. The blocking effects of anti–adhesion molecule antisera on the lymphocyte binding were estimated by quantitative analysis of binding cells with a Nexus 600 image processor, as mentioned above.

Statistical Analysis. For statistical analyses, Student's *t*-test was employed in this study.

RESULTS

Lymphocyte Binding to Various Mucosae and Salivary Glands. Figure 1 illustrates the mean numbers of lymphocytes bound to various mucosae and salivary glands. Lymphocytes bound to 20 sections of various tissues were counted at 400× magnification with an image processor. Although lymphocytes of PPs and spleen from animals with AMI or ASI were hardly observed binding with mucosae of the control ear and trachea, those cells bound with mucosae of the inflamed middle ear, nose, and small intestine, plus salivary gland. Many PP lymphocytes from animals with AMI, which were rich in ASAFCs, bound with the inflamed middle ear mucosa, nasal and small intestinal mucosae, and salivary glands. The mean numbers of these binding cells were significantly greater than those of animals with ASI in which ASGFCs were induced in the spleen ($p < .001$). Also, PP lymphocytes from animals with AMI bound with the inflamed middle ear mucosa and small intestine were significantly greater than those of spleen cells from those animals ($p < .001$). However, mean values of PP and spleen lymphocytes bound with the nasopharyngeal mucosa and trachea were not significantly different between the animals with AMI and those with ASI ($p > .001$).

Influences of Activated Mucosal Immunity on Lymphocyte Binding to Inflamed Middle Ear Mucosa. Table 1 summarizes the mean numbers of PP and spleen lymphocytes from animals with AMI or ASI bound with middle ear mucosae inflamed with antigenic and nonantigenic stimuli. Regardless of anti-

TABLE 1. MEAN NUMBERS OF BINDING LYMPHOCYTES OF PEYER'S PATCHES AND SPLEEN FROM ANIMALS WITH AMI OR ASI TO INFLAMED MIDDLE EAR MUCOSA

		Inflamed Middle Ear Mucosa	
Source		*Antigenic Stimulation (n = 20)*	*Nonantigenic Stimulation (n = 20)*
Peyer's patches	With AMI	139 ± 31[a]	123 ± 19[e]
	With ASI	85 ± 13[b]	83 ± 23[f]
Spleen	With AMI	60 ± 14[c]	62 ± 20[g]
	With ASI	57 ± 13[d]	52 ± 14[h]

a > b, c, and d ($p < .001$); e > f, g, and h ($p < .001$).

AMI — activated mucosal immunity, ASI — activated systemic immunity.

genic and nonantigenic stimuli to the middle ear mucosa, the mean numbers of binding PP lymphocytes from animals with AMI or ASI were significantly greater than those of spleen lymphocytes from those animals ($p < .001$). Furthermore, the number of binding cells of PPs from animals with AMI was significantly greater than that of PPs from animals with ASI ($p < .001$), while the binding of spleen cells was not statistically different between animals with AMI or ASI ($p > .001$).

Binding of Various Lymphocyte Fractions From Animals With Mucosal Immunity to Inflamed Middle Ear Mucosa. Table 2 summarizes the mean numbers of binding cells of various lymphocyte fractions from PPs and spleen with AMI to the middle ear mucosa. The mean value of the IgG cell fraction from PP was significantly smaller than those of whole cell, B cell, and IgA cell fractions ($p < .001$), while there were not significant differences of the binding cell numbers among whole cell, B cell, and IgA cell fractions of PPs ($p > .001$).

Histologic Distribution of Binding Lymphocytes to Various Mucosae and Salivary Glands. Many lymphocytes of PPs from animals with AMI were observed bound with endothelial cells of newly grown vessels in the inflamed middle ear mucosa (Fig 2A). Lymphocytes of both PPs and spleen from animals with AMI or ASI bound mainly to endothelial cells of vessels on the respiratory and intestinal mucosae. A few lymphocytes were observed bound to the basal lamina of the epithelial and acinar cells on the respiratory and intestinal mucosae (Fig 2B,C). On the salivary gland, those cells bound to the basal portion of the acinar and ductal cells (Fig 2D). However, few lymphocytes were observed bound to the submucosal connective tissue of various mucosae and interglandular connective tissue of the salivary gland. Lymphocytes of PPs from animals with AMI tended to bind to the respiratory mucosae more than spleen lymphocytes from those animals or lymphocytes of PPs and spleen from animals with ASI.

Distribution of Various Adhesion Molecules on Human Middle Ear Mucosa. In the inflamed middle ear mucosa, newly grown vessels were frequently found, and their endothelial cells were bound to both T and B lymphocytes of peripheral blood. Those endothelial cells were strongly stained by anti–ICAM-1 antibody (Fig 3A), but the staining of those cells by anti–VCAM-1 (Fig 3B), anti–ELAM-1 (Fig 3C), and anti-PECAM antibodies (Fig 3D) was weaker than that of anti–ICAM-1 antibody. The basal portion and basal lamina of the epithelium and fibroblastlike cells with a large nucleus, as well as vascular endothelial cells (Fig 4A), were also stained by anti–ICAM-1 (Fig 4B) and anti-PECAM antisera. A few lymphocytes were also observed bound with those cells.

Influences of Anti–Human Adhesion Molecule Antibodies on Lymphocyte Binding. Table 3 summarizes mean numbers of binding lymphocytes to human middle ear mucosa after treatments with various anti–human adhesion molecule antibodies. When

TABLE 2. BINDING OF VARIOUS LYMPHOCYTE FRACTIONS OF PEYER'S PATCHES AND SPLEEN FROM ANIMALS WITH ACTIVATED MUCOSAL IMMUNITY TO INFLAMED MIDDLE EAR MUCOSA

		Inflamed Middle Ear Mucosa	
Source	*Lymphocyte Fraction*	*Antigenic Stimulation (n = 20)*	*Nonantigenic Stimulation (n = 20)*
Peyer's patches	Whole fraction	100 ± 23[a1]	100 ± 16[a2]
	B cell fraction	87 ± 10[b1]	93 ± 13[b2]
	IgA cell fraction	86 ± 17[c1]	87 ± 22[c2]
	IgG cell fraction	15 ± 7[d1]	37 ± 13[d2]
Spleen	Whole fraction	100 ± 29[e1]	100 ± 26[e2]
	B cell fraction	93 ± 14[f1]	92 ± 15[f2]
	IgA cell fraction	88 ± 20[g1]	88 ± 21[g2]
	IgG cell fraction	78 ± 15[h1]	83 ± 23[h2]

Data are percentages: (Binding cells of various fractions)/(Mean number of binding cells of whole fraction) × 100.

d1 and d2 < a1, a2, b1, b2, c1, and c2 ($p < .001$).

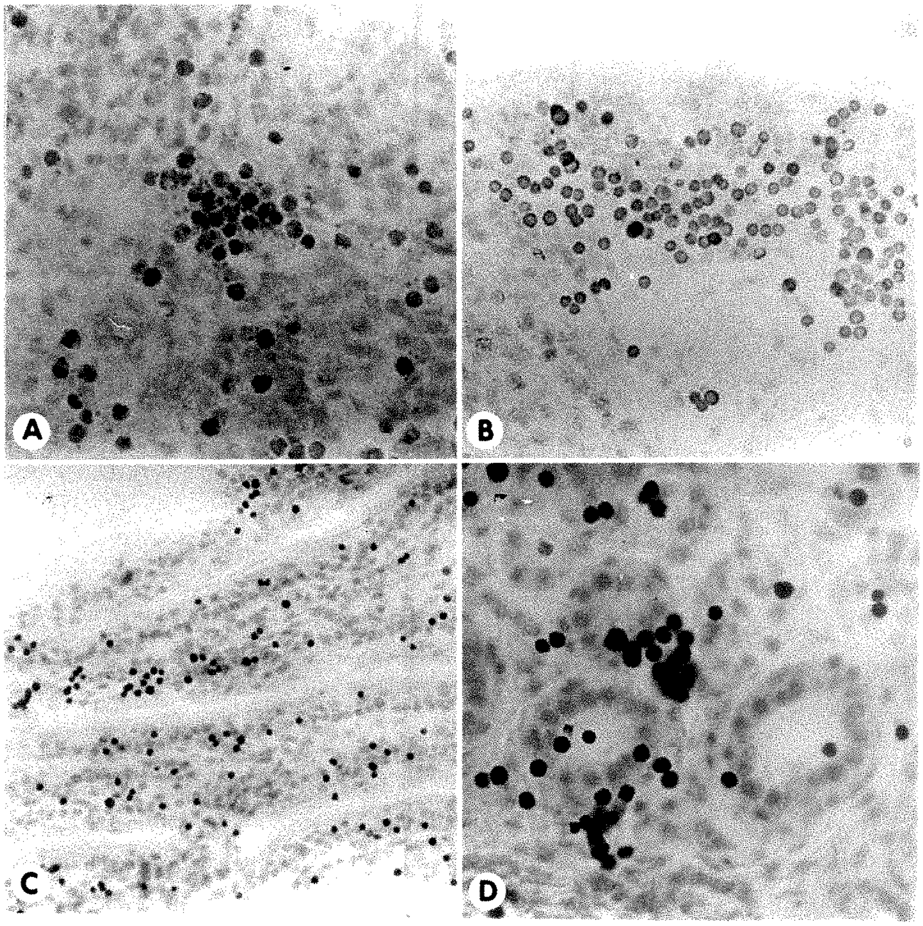

Fig 2. Binding lymphocytes of PPs from animals with AMI to various mucosae (original ×400). A) Many lymphocytes of PPs from animals with AMI are observed bound with endothelial cells of newly grown vessels in inflamed middle ear mucosa. B) Many lymphocytes of PPs from animals with AMI are found to bind with basal portion of pseudostratified ciliated epithelial cells of nasal mucosa. C) Many lymphocytes are observed bound to basal lamina of epithelial and acinar cells of intestinal mucosae. D) Lymphocytes of PPs from animals with AMI are scattered around acinar cells of nasal mucosa.

anti–ICAM-1 antibody was applied to tissue sections, binding cells were significantly decreased compared to those sections treated with anti–VCAM-1, anti-PECAM, and anti–ELAM-1 antibodies and those without antibody treatment ($p < .001$). However, there were not significant differences of binding cell numbers among tissue sections treated with anti–VCAM-1, anti-PECAM, and anti–ELAM-1 antibodies and without antibody treatment ($p > .001$).

DISCUSSION

Although many lymphocytes of PPs from animals with AMI bound with the inflamed middle ear mucosa, those cells were hardly observed to bind with middle ear mucosae of the control ear without inflammation. This finding corresponds with our previous results.[11,16,17] The middle ear cavity is usually separated from antigenic environments, and antigenic substances can enter into the middle ear cleft when the eustachian tube is opening. So, immune-competent cells are hardly found in the normal middle ear mucosa, but many IgA-forming cells appear in the inflamed middle ear mucosa. Therefore, the middle ear mucosa may be an effector site of the mucosal immunity, when the mucosa is stimulated by antigenic and inflammatory substances.

The influence of antigenic stimulation on the migration of ASAFCs is controversial. Husband[18] demonstrated that the extravasation of antigen-specific IgA-containing cells into the gut lamina propria was antigen-independent, while these cells only persisted in the mucosal site if the antigen was administered on the mucosal surface. However, our previous study[17] has demonstrated that at the early stage of inflammation, circulating lymphocytes migrate nonspecifically to the inflamed middle ear mucosa by the inflammatory reaction by which the antigen-specific cell migration may be masked. As shown in the present study, the binding of PP lymphocytes from animals with AMI or ASI to the inflamed middle ear mucosa was significantly greater than that of spleen cells from those animals, and this binding was not affected by antigenic and nonantigenic stimuli to the middle ear mucosa. This finding is consistent with our previous results[17] and those of Ryan et al.[19] Therefore, the influence of antigen on lymphocyte migration to the inflamed middle ear mucosa may be smaller than that of the inflammation, even if it exists.

Other studies have suggested that the interactions between various adhesion molecules on vascular endothelial cells, and their ligands on lymphocytes, may play an important role in migration from the

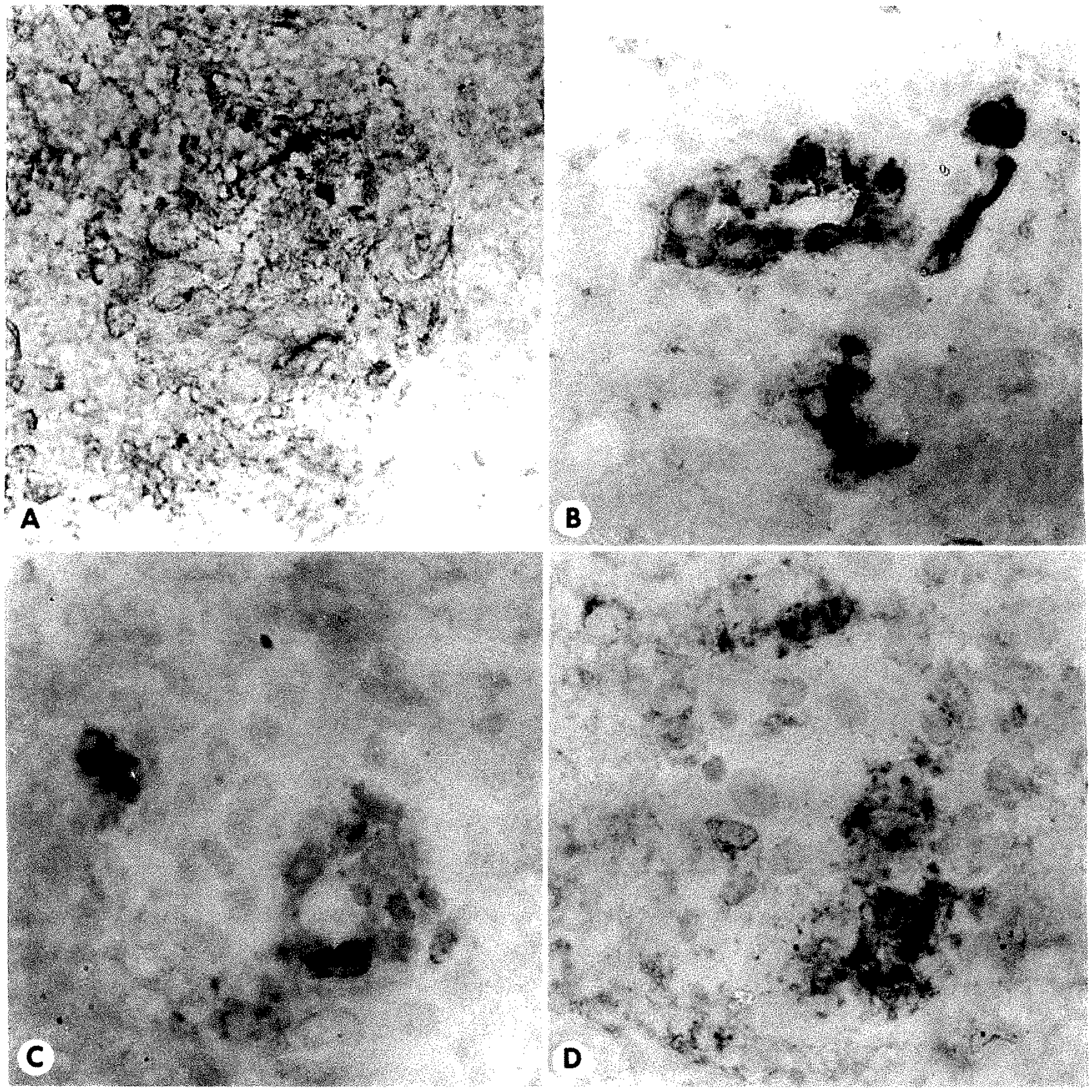

Fig 3. Serial frozen sections from human middle ear mucosa were stained with various monoclonal antibodies against human adhesion molecules by avidin-biotin complex technique (original ×400). In inflamed middle ear mucosa, newly grown vessels were strongly stained with anti–ICAM-1 antibody (A). Staining of those cells by anti–VCAM-1 (B), anti–ELAM-1 (C), and anti–PECAM-1 (D) antibodies was weaker than that by anti–ICAM-1 antibody.

systemic circulation to the local sites.[4-8] At the initial stage of cell migration, cells are thought to adhere to endothelial cells and travel with those cells along the blood flow. The selectin family, including leukocyte–endothelial cell adhesion molecule (LECAM)–1 on lymphocytes and endothelial-leukocyte adhesion molecule (ELAM)–1 on endothelial cells, bind with sialic acid and saccharide chains on the cell surface. These bindings are thought to participate in the rolling process.[20,21] Cells rolling on endothelial cells may more firmly adhere to endothelial cells by interaction between various adhesion molecules on endothelial cells, such as ICAM-1, VCAM-1, PECAM, and ELAM-1 and their ligands on lymphocytes. They then extravasate into the extravascular space of local sites from the luminar space of blood vessels.[22] In the inflammatory reaction, those adhesion molecules are expressed on endothelial cells and other cells by inflammatory cytokines, such as interleukin-1β, tumor necrosis factor α, and interferon-γ.[23] As demonstrated in the present study, ICAM-1, VCAM-1, PECAM, and ELAM-1 are present on endothelial cells of newly grown vessels in the inflamed human middle ear mucosa. Also, anti–ICAM-1 antibody significantly suppressed lymphocyte binding to the inflamed middle ear mucosa. These findings indicate that various adhesion molecules are expressed on vascular endothelial cells in the inflamed middle ear mucosa and that ICAM-1 may play an important role in lymphocyte extravasation from the systemic circulation to the inflamed middle ear mucosa.

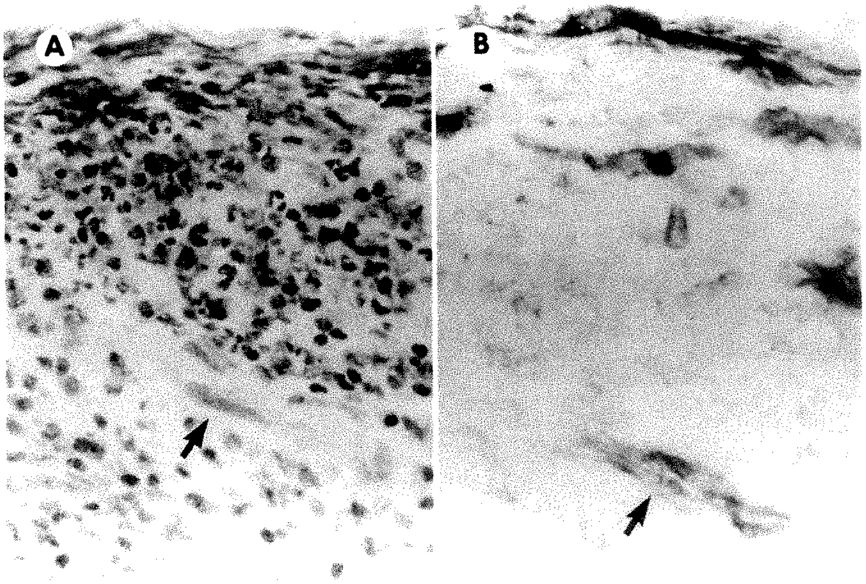

Fig 4. Serial sections of human middle ear mucosa stained with A) hematoxylin and eosin and B) anti–ICAM-1 antiserum (original ×400). Basal portion of epithelium and fibroblastlike cells (arrow) with large nucleus (A) are also stained by anti–ICAM-1 (B).

The interaction between adhesion molecules on endothelial cells and their receptors on lymphocytes may account for the extravasation of lymphocytes from the systemic circulation to the mucosal sites, but not for their retention and lodging in the local sites. O'Sullivan and Montgomery[9] suggested the presence of organ-specific adhesion factors on the lacrimal gland, and we also reported similar results on the inflamed middle ear mucosa.[11] In the present study, PP lymphocytes more frequently bound with the inflamed middle ear mucosa than did those of spleen cells. Some lymphocytes were observed bound to tissue components such as acinar cells and basement lamina, different from vascular endothelial cells, on the various mucosae and salivary glands. Furthermore, although anti–ICAM-1 antibody significantly suppressed PP cell binding with the inflamed middle ear mucosa, antibodies against various adhesion molecules could not completely inhibit that binding. These findings suggest that organ-specific adhesion factors different from endothelial adhesion molecules are present in the inflamed middle ear mucosa and play an important role in retention and lodging at the local site.

As shown in the present study, PP lymphocytes from animals activated by mucosal immunization more frequently bound with the inflamed mucosa, while this binding was not affected by antigenic or nonantigenic stimuli to the middle ear mucosa. Mucosal immunity is activated by mucosal immunization of a particulate antigen, and PP lymphocytes from animals with AMI are rich in ASAFCs, as previously reported.[1] These findings indicate that ASAFCs more frequently bind with inflamed middle ear mucosa than nonactivated PP cells and ASGFCs in spleen from animals with ASI. Thus, after enhancement of the mucosal immunity, ASAFCs are induced in PP, and those activated cells frequently migrate to the inflamed middle ear mucosa through the systemic circulation, regardless of antigenic and nonantigenic stimuli to the mucosa. In this migration, the activation of PP lymphocytes seems to be more important than other factors such as antigenic stimulation to the local site. Antigenic and nonantigenic stimuli induce an inflammation on the middle ear mucosa and activate new expressions of various adhesion molecules on endothelial cells of newly grown vessels in inflamed mucosa.[24] On the other hand, the activation of mucosal immunity activates IgA precursors and induces their differentiation. The stimulation of IgA cell activation, including anti-

TABLE 3. MEAN NUMBERS OF LYMPHOCYTES BINDING TO HUMAN MIDDLE EAR MUCOSA AFTER TREATMENTS WITH VARIOUS ANTI–HUMAN ADHESION MOLECULE ANTIBODIES

	Antibody Treatment				
Lymphocyte Fraction	*Anti–ICAM-1*	*Anti–VCAM-1*	*Anti-PECAM*	*Anti–ELAM-1*	*Control*
Whole fraction (n = 10)	19 ± 6^a	75 ± 13^b	60 ± 16^c	70 ± 19^d	76 ± 24^e
B cell fraction (n = 10)	48 ± 9^f	66 ± 14^g	72 ± 10^h	68 ± 11^i	70 ± 14^j

a < b, c, d, e, and f ($p < .005$); f < g, h, i, and j ($p < .005$).

genic stimuli, may also induce a new expression of receptors and ligands on cell surfaces.[25,26] Those newly expressed receptors on IgA cells could be one of the more important factors in the lymphocyte migration.

Murakami et al[27] demonstrated that B cells activated by interleukin-5 bound strongly to a hyaluronate-coated surface, and that this binding was specifically inhibited by anti–Pgp-1 (CD44) antibody. Pgp-1 (CD44) is known to be an 80 to 95 kd cytoskeleton-associated cell surface glycoprotein on hemopoietic cells, myeloid cells, lymphocytes, and nonhematolymphoid cells.[28,29] Hyaluronate is widely distributed in extracellular matrices. In the inflammatory site, antigen-stimulated helper-T cells secrete interleukin-5. Interleukin-5 activates B cells to express Pgp-1 (CD44) on their surfaces, and activated B cells bind to hyaluronate on cell surfaces and extracellular matrices. Furthermore, Dang and Rock[30] also reported that the stimulation of surface immunoglobulin receptors on B cells with anti-IgM antibodies results in a marked increase of adhesion molecule expression on their cell surfaces, including leukocyte function–associated antigen 1 and ICAM-1. These findings are consistent with our results, in which activated PP lymphocytes more frequently bound with the inflamed mucosa. The activation of B cells with antigen results in increased and/or new expression of binding factors on their surfaces, including adhesion molecules and other adhesion factors, and those binding factors may contribute to the local lodging and retention in the inflammatory site after extravasation.

In conclusion, PP lymphocytes from animals with activated mucosal immunity more frequently migrate to the inflamed middle ear mucosa. This finding indicates that the activation of PP lymphocytes results in new and/or increased expression of some receptors on those cells. Those receptors expressed on activated cells may bind with organ-specific binding factors on the inflamed mucosa, different from adhesion molecules on vascular endothelial cells, and through this binding, play an important role in the local lodging and retention of B cells in local sites after extravasation. Of course, immunochemical and immunohistochemical studies are necessary to describe the immunologic and immunochemical features of those organ-specific binding factors on the inflamed mucosa and their receptors on activated PP lymphocytes. In regard to lymphocyte migration, we should consider not only the expression of various adhesion molecules on vascular endothelial cells, but also the activation of B cells for new and/or increased expression of binding receptors on their surfaces.

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FOURTH ANNUAL GOLF SEMINAR — ADVANCES IN OTOLARYNGOLOGY–HEAD AND NECK SURGERY

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Ann Otol Rhinol Laryngol 105:1996

BASEMENT MEMBRANE IN MIDDLE EAR CHOLESTEATOMA

IMMUNOHISTOCHEMICAL AND ULTRASTRUCTURAL OBSERVATIONS

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We investigated the distribution of basement membrane zone (BMZ) components collagen type IV, collagen type VII, and fibronectin in human middle ear cholesteatoma, auditory meatal skin, and middle ear mucosa using both immunohistochemical and ultrastructural methods. Collagen type IV immunoreactivity of skin and middle ear mucosa is continuous in the BMZ, whereas cholesteatoma frequently showed absent immunoreactivity or focal discontinuities. Collagen type VII immunoreactivity is detected similarly within the BMZ of cholesteatoma and skin. Fibronectin immunoreactivity is observed within the dermoepithelial junction of skin and middle ear mucosa. In cholesteatoma, however, fibronectin immunoreactivity is markedly increased within the extrinsic BMZ and the subepithelial connective tissue. The ultrastructural arrangement of the BMZ of cholesteatoma is like that of skin; however, it exhibits distinct alterations of the lamina fibroreticularis and lamina densa. Our results outline cholesteatoma as a disease with disturbed cell matrix interactions analogous to those of wound reepithelialization.

KEY WORDS — basement membrane, cholesteatoma, collagen type IV, collagen type VII, electron microscopy, fibronectin, immunohistochemistry, LH7.2.

INTRODUCTION

By light microscopy the basement membrane zone appears as a well-defined, thin, glycoprotein-rich layer immediately below the epithelium. The basement membrane zone is a ubiquitous extracellular structure that anchors the squamous epithelium to the subepithelial connective tissues but simultaneously separates these two layers.[1] Its complex structure is important for the modulation of epithelial expansion and the maintenance of cutaneous function.[2] As a highly heterogeneous network formed by collagens and elastin, its components are embedded in a viscous ground substance composed of proteoglycans, glycoproteins, collagens, and noncollagenous adhesion molecules like laminin and fibronectin.[3,4]

Collagen type IV is a major component of the basement membrane in the dermal-epidermal junction.[5] It is a nonfibrillar collagen with a molecular weight of 550,000 to 600,000 d and constitutes the structural scaffolding of specialized sheets of extracellular matrix.[6] Increased attention has been focused on the role of collagen type IV as a stabilizing protein of extracellular matrix in the process of tumor invasion and metastasis.[7,8]

Type VII collagen is a recently characterized collagen that appears to be the major component of the anchoring fibril in the sub–lamina densa zone occurring as an antiparallel dimer.[9] This long-chain 510,000 d collagen molecule forms parallel lateral alignments, and the overlapping of the amino termini produces end-on-end dimers.[10] Leigh et al[11] produced a monoclonal antibody, LH7.2, that reacts with the carboxy terminus of type VII collagen.

Fibronectin, a noncollagenous glycoprotein of about 440,000 d molecular weight, is found as an insoluble multimer in cells and as a soluble dimer in plasma.[12] Fibronectin plays an important role in wound healing: it promotes the spreading of platelets at the site of injury; the adhesion and migration of neutrophils, monocytes, fibroblasts, and endothelial cells into the wound region; and the migration of epidermal cells through the granulation tissue.[13] At the level of matrix synthesis, fibronectin appears to be involved in both the organization of the granulation tissue and the assembly of the basement membrane.[14]

The purpose of the present investigation on middle ear cholesteatoma was to examine the occurrence,

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distribution, and arrangement of intrinsic and extrinsic basement membrane components by means of immunohistochemical methods and electron microscopy. We focused on the basement membrane zone in order to delineate its possible role in the pathogenesis of cholesteatoma and tried to elucidate the modified keratinocyte behavior observed in this disease.[15,16]

MATERIAL AND METHODS

Specimens were obtained from patients requiring middle ear surgery. Thirty cholesteatomas, 7 samples of normal auditory canal wall skin, and 5 samples of normal middle ear mucosa were analyzed with respect to expression of extracellular matrix components. Some samples was embedded in Tissue Tek (Miles, Elkhart, Ind) and rapidly frozen in liquid nitrogen. Cryosections of 4 µm were cut at −20°C, mounted on poly-L-lysine–coated slides, and prepared for the alkaline phosphatase–anti–alkaline phosphatase (APAAP) immunostaining procedure. Other samples were fixed in formalin, embedded in paraffin, sectioned at 4 µm, and labeled by the avidin-biotin complex (ABC) method. All specimens were examined histopathologically with routine hematoxylin and eosin staining. Sections from 6 cholesteatomas were prepared for ultrastructural investigations by transmission electron microscopy.

Antibodies. The following polyclonal and monoclonal antibodies were used:

1. Affinity-purified polyclonal mouse anti human antibody against collagen type IV (Dianova, Hamburg, Germany) at a dilution of 1:40.
2. Anti-human type VII collagen monoclonal antibody, undiluted (gift of Dr I. M. Leigh, Experimental Dermatology Research Laboratory, London Hospital, London, England).
3. Mouse monoclonal antibody (Böhringer-Mannheim, Mannheim, Germany) directed against the fibronectin cell-binding fragment (FCBF) at a dilution of 1:5.

Avidin-Biotin Complex Method. Immunohistochemical staining was performed by using the ABC technique (Vectastain, Camon Laboratories, Wiesbaden, Germany) according to the method described by Hsu et al.[17] Serial 4-µm paraffin sections were placed on poly-L-lysine-coated slides (10% poly-L-lysine; Sigma, Deisenhofen, Germany), deparaffinized, hydrated, and dried overnight at 37°C. Polyclonal antibody against collagen type IV was diluted with phosphate-buffered saline (PBS, pH 7.4) containing 0.1% bovine serum albumin. Endogenous peroxidase activity was eliminated with 3% hydrogen peroxide at room temperature for 10 minutes and nonspecific protein binding by incubation with 10% normal goat serum at 20°C for 20 minutes. After washing with PBS, the sections were incubated for 30 minutes with a secondary biotinylated rabbit anti mouse IgG antibody, diluted 1:200 (Vectastain-Kit, Camon Laboratories), followed by incubation with a complex of avidin and biotinylated peroxidase over 30 minutes at room temperature and development with 3-amino-9-ethylcarbazole (Sigma), yielding a red precipitate. Sections were counterstained with Mayer's hematoxylin, dehydrated, and mounted with synthetic mounting medium. Preimmune sera and the omission of the primary antibody were used as negative controls. Immunohistochemical demonstration of collagen type IV in endothelial basement membrane served as a positive internal control.

Alkaline Phosphatase–Anti–Alkaline Phosphatase Method. Monoclonal antibodies directed against collagen type VII and the FCBF were used as primary antisera. The bridging antibody (rabbit anti mouse IgG) and the APAAP complex were purchased from Dako (Hamburg, Germany) and used in a dilution of 1:50 in 5% normal pig serum (Dako), respectively. The tissue sections were fixed with acetone for 10 minutes and treated with pig serum at a dilution of 1:5 in PBS (pH 7.4) for 20 minutes in order to block nonspecific bindings. The sections were then incubated with the primary monoclonal antibody in a moist chamber at room temperature for 30 minutes, washed, and treated with the bridging antibody and the APAAP complex for 30 minutes, respectively. The enzyme-linked antibody was stained with the fuchsin procedure described by Cordell et al.[18] Each tissue section was then lightly counterstained with Mayer's hematoxylin.

Tissue specimens were examined and photographed with a Zeiss Axiophot light microscope. Immunoreactivity was designated as continuous or discontinuous by independent observers.

Transmission Electron Microscopy. Tissue samples were fixed in 3.5% glutaraldehyde with 0.05 mol/L sodium phosphate buffer (pH 7.4) at 4°C for 1 hour, post-fixed in 1% osmium tetroxide (pH 7.4) for 2 hours, and washed three times for 5 minutes with PBS. The tissue was dehydrated with ethanol and propylene oxide and embedded in Durcupan (Balzers, Walluf, Germany). Semithin sections (1 µm) were stained with toluidine blue for preliminary orientation. Ultrathin sections (60 to 80 nm) were stained with uranyl acetate and lead citrate for electron microscopic investigations (Zeiss 910, 50 to 80 kV).

RESULTS

Collagen Type IV. The ABC stain for collagen type

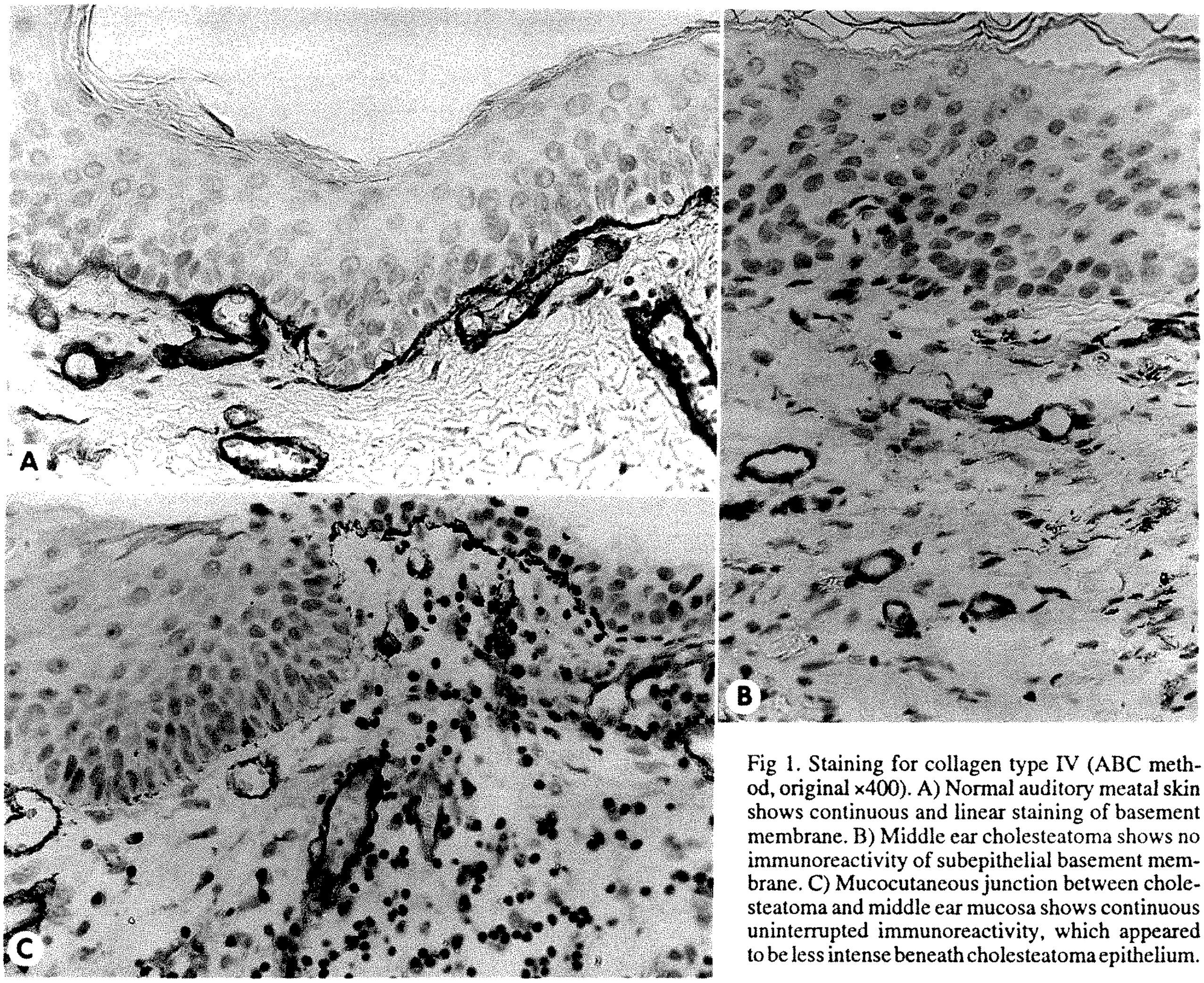

Fig 1. Staining for collagen type IV (ABC method, original ×400). A) Normal auditory meatal skin shows continuous and linear staining of basement membrane. B) Middle ear cholesteatoma shows no immunoreactivity of subepithelial basement membrane. C) Mucocutaneous junction between cholesteatoma and middle ear mucosa shows continuous uninterrupted immunoreactivity, which appeared to be less intense beneath cholesteatoma epithelium.

IV showed a continuous linear immunoreactivity within the basement membrane of normal auditory meatal skin and middle ear mucosa (Fig 1A). No collagen type IV immunostaining of the subepithelial basement membrane could be found in 13 of 30 investigated cholesteatomas, with the positive immunoreactivity of the basement membranes of adjacent subepithelial blood vessels taken as a positive internal control (Fig 1B). The 17 cases of positive immunoreactivity for collagen type IV revealed a distinct linear staining of the intrinsic basement membrane, exhibiting focal discontinuities, especially in areas of intense subepithelial inflammation. Within the mucocutaneous junction between the cholesteatoma and middle ear mucosa there was a continuous uninterrupted immunoreactivity for collagen type IV antibody, which appeared to be less intense beneath cholesteatoma epithelium (Fig 1C).

Collagen Type VII. Immunolabeling for collagen type VII with LH7.2 revealed a continuous linear staining of the intrinsic basement membrane zone in samples taken from normal auditory meatal skin. The distribution of collagen type VII immunoreactivity in the basement membrane zone of cholesteatoma (Fig 2) showed a pattern similar to that found in auditory meatal skin, whereas middle ear mucosa and the basement membranes of blood vessels of the underlying connective tissue exhibited no immunoreactivity. Epithelial cells of middle ear mucosa, skin, and cholesteatoma also showed no reactivity with LH7.2 (Fig 2).

Fibronectin. The FCBF immunolabeling revealed a continuous linear pattern within the extrinsic basement membrane zone of auditory meatal skin and middle ear mucosa. No specific immunoreactivity for fibronectin was observed in either type of epithelium. In middle ear cholesteatoma, fibronectin immunoreactivity was remarkably increased within the extrinsic basement membrane zone, the adjacent subepithelial connective tissue (Fig 3), and granulation tissue. Fibronectin immunoreactivity was noticed on the surface of collagen fibrils, endothelial cells of blood vessels, fibroblasts, and macrophages. Serial dilutions of the primary antibody showed a progres-

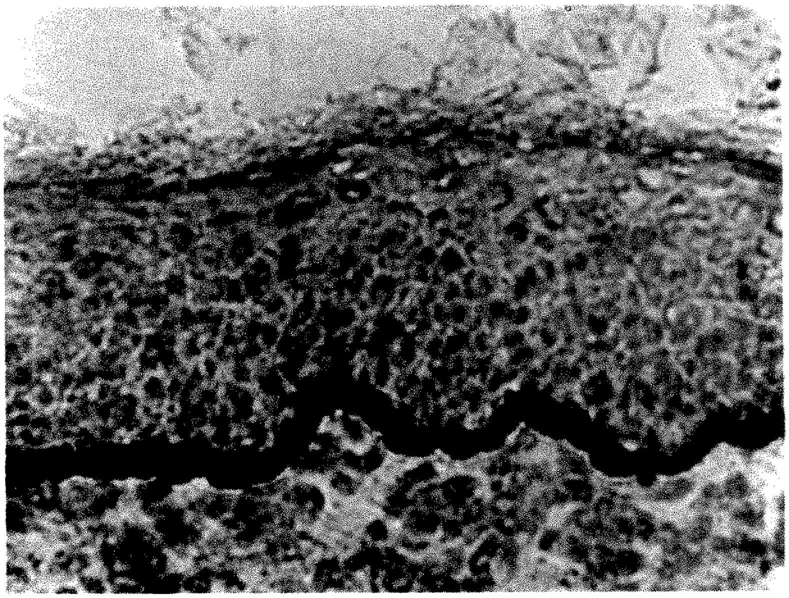

Fig 2. Distribution of collagen type VII immunoreactivity in basement membrane zone of cholesteatoma (APAAP method, original ×400).

sive loss of the immunoreactivity within the underlying connective tissue, whereas the prominent immunoreactivity of the dermoepidermal junction remained. A reticular staining pattern was observed within the stroma of cholesteatoma, frequently showing regions of different intensities of immunoreactivity. The epithelium of cholesteatoma showed no positive immunoreactivity (Fig 3).

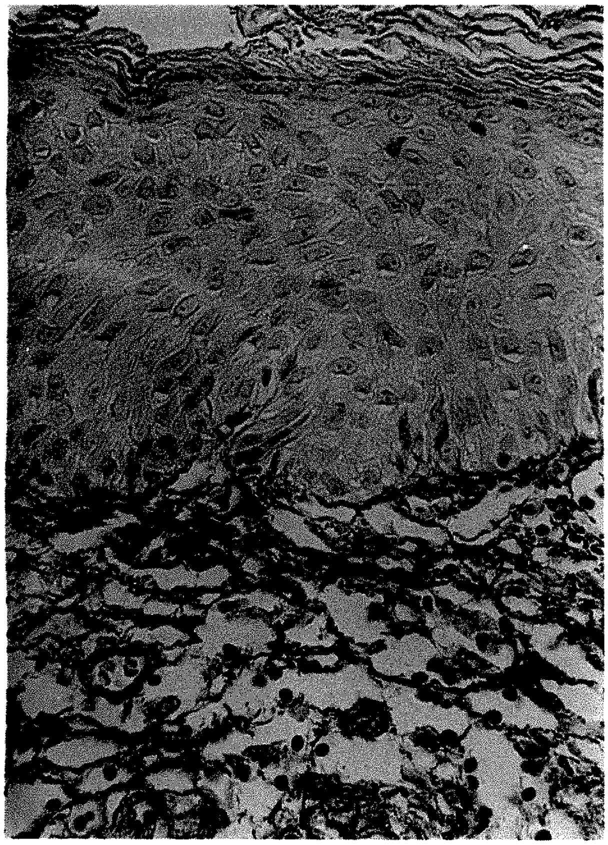

Fig 3. Increased fibronectin immunoreactivity within extrinsic basement membrane zone and adjacent subepithelial connective tissue of cholesteatoma (APAAP method, original ×400).

Electron Microscopy. The basement membrane of middle ear cholesteatoma exhibited two main layers: the electron-lucent lamina rara or "lucida," and the electron-dense "lamina densa" (Fig 4A). We found the thickness of the lamina rara to be between 0.03 and 0.08 μm, and of the lamina densa, between 0.04 and 0.11 μm. The keratinocyte basal cell membrane displayed hemidesmosomes with a locally distinct number and structure. Within this area a marked increase of tonofilaments was observed, radiating from the basis of the basal cells to the lamina densa. We observed an increased deposition of collagen fibers beneath the epithelial basement membrane, displaying atypical fibrillar aggregation. The lamina fibroreticularis of cholesteatoma revealed anchoring fibrils and scattered amorphous material of irregularly arranged collagen fibrils and fine fibrils. Cytoplasmic protrusions, leading to basement membrane irregularities, reduplications, and focal discontinuities, were discovered within the lamina densa (Fig 4B).

DISCUSSION

Middle ear cholesteatoma is histopathologically characterized as keratinizing stratified squamous epithelium growing in foreign sites within the middle ear cavity and frequently associated with subsequent bone destruction.[19] In cases of secondary cholesteatoma, the epithelium originates from basal cells of the tympanic membrane or the drum-adhering meatal skin. In auditory meatal skin, basal cells are in contact with the basement membrane and eventually detach from this structure, move vertically, and differentiate. In contrast, during cholesteatoma development

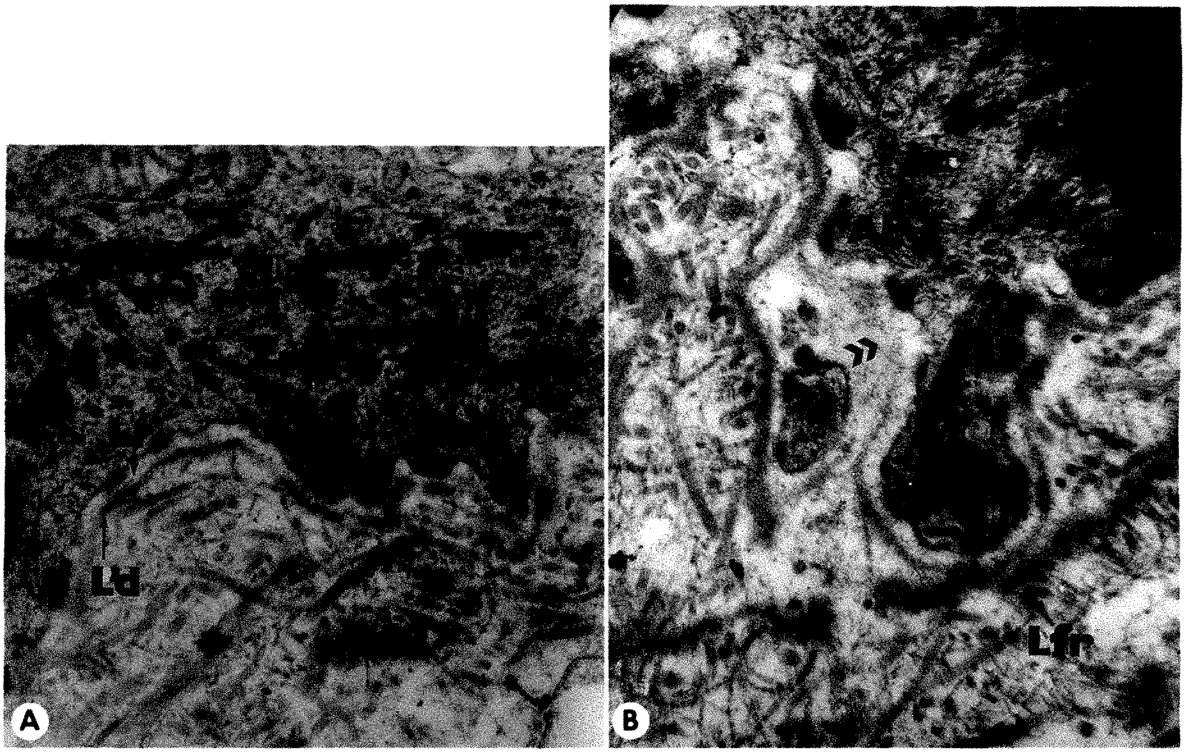

Fig 4. Electron microscopy (original ×30,000). A) Basement membrane of middle cholesteatoma consists of two main layers: electron-lucent lamina rara (Lr) or "lucida," and electron-dense "lamina densa" (Ld). B) Lamina fibroreticularis (Lfr) of cholesteatoma reveals anchoring fibrils (AF) and scattered amorphous material of irregularly arranged collagen fibrils and fine fibrils. Also shows cytoplasmic protrusions (P) and reduplications and focal discontinuities (double arrowhead) of lamina densa.

the basal cells migrate laterally to substitute for the middle ear mucosa and remain in a hyperproliferative state.[15,16,20]

Eight of the investigated cholesteatoma samples showed the mucocutaneous junction between the cholesteatoma epithelium and middle ear mucosa. Within those samples we found a continuous immunoreactivity for collagen type IV beneath both epithelia, which could indicate that the advancing cholesteatoma epithelium migrates into the middle ear cavity, utilizing the original middle ear mucosa basement membrane as a guide. Our results showed a continuous immunoreactivity for collagen type IV of the basement membrane within less-inflamed cholesteatoma samples. Conversely, the more intensely inflamed cholesteatoma samples, as well as epithelial cones infiltrating the underlying connective tissue, showed discontinuous basement membranes and lacked collagen type IV immunoreactivity. Ergün et al[21] described a lack of immunostaining in some parts of the basement membrane in 1 of 6 cholesteatomas investigated. The lack of immunoreactivity found by Ergün et al and by us probably indicates an incomplete assembly or diminished turnover of this protein. Disruptions associated with inflammation are presumably linked to the release of proteolytic enzymes, like metalloproteinases, which are known to be released during inflammation.[8,22] One of these proteinases, type IV collagenase, is destructive and able to degrade the type IV collagen network.[22]

Type VII collagen appears to be a target for some diseases of the dermoepidermal junction.[23] The reaction with LH7.2 for collagen type VII is missing in tissues with recessive dystrophic epidermolysis bullosa (RDEB), a hereditary disease in which anchoring fibrils are defective.[22] Our inability to find any disruptions or alterations with LH7.2 immunoreactivity of cholesteatoma's basement membrane suggests no major alterations of anchoring fibrils. The similar immunoreactivity between auditory meatal skin and cholesteatoma epithelium and the absent immunoreactivity in middle ear mucosa and the basement membrane of blood vessels imply that LH7.2 immunoreactivity is associated with a stratified squamous epithelial phenotype. The LH7.2 epitope is resistant to bacterial collagenase and to collagenase from RDEB fibroblasts[11] and was not found to be degraded in cholesteatoma.

Fibronectin is a structural glycoprotein widely deposited in chronic inflammatory lesions.[24] It is one of the early macromolecules occurring in wound healing, is an important protein in wound repair,[25] and promotes phagocytosis of particles by macrophages and fibroblasts.[26] Fibronectin is known to mediate the adhesion of fibroblasts and endothelial cells in vitro[27] and to promote the migration and proliferation of keratinocytes in vitro.[28] Therefore, it must be considered to have an important role in the pathogenesis of cholesteatoma. Comparison of epithelial healing in skin and cornea reveals features common to cholesteatoma development, as well as significant differences: with time, the repaired epidermis loses its expression of fibronectin. Cholesteatoma, however, exhibits significant quantities of fibronectin within the inflamed subepithelial connective tissue and granulation tissue. As synthesis and deposition of fibronectin persist, cholesteatoma could be interpreted as a nonhealing wound.

The ultrastructural analysis of the basement membrane of cholesteatoma confirms the results found by Chole and Tinling[29] and Wright et al[30] in experimentally induced cholesteatomas. Both described breakthroughs, fragmentations, and cytoplasmic protrusions of the basement membrane. We also could find focal discontinuities of the basement membrane of cholesteatoma, which could be due to the release of proteolytic enzymes.[22] The increased deposition of collagen beneath the epithelial basement membrane and the scattered amorphous material within the lamina fibroreticularis of cholesteatoma probably result from increased collagen synthesis during chronic inflammation and an enhanced degradation of extracellular matrix components.[31]

CONCLUSIONS

Considering the alterations in the assembly of the basement membrane, we regard cholesteatoma as a hyperproliferative and chronic inflammatory middle ear disease. It exhibits behaviors analogous to regenerative processes like wound healing. This report suggests that a combined analysis of extracellular matrix components may be a useful tool for the evaluation of the clinical behavior of this middle ear disease. Further studies on the extracellular matrix, including investigations on the release of proteolytic enzymes, especially matrix metalloproteinases, in cholesteatoma are necessary for a better understanding of its pathogenesis and an improved treatment.

ACKNOWLEDGMENT — We thank K. Lempart for excellent technical assistance.

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Ann Otol Rhinol Laryngol 105:1996

ACOUSTIC RHINOMETRY IN THE STUDY OF THE ACUTE NASAL ALLERGIC RESPONSE

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Acoustic rhinometry is a recently developed method for the objective assessment of nasal patency. In this study, acoustic rhinometry was used to measure changes in nasal cavity dimensions in the immediate response to nasal allergen challenge in eight pollen-sensitive subjects. Acoustic rhinometric changes were compared with subjective symptoms, as well as histamine in nasal secretions, cytology of nasal mucosal scrapings, and changes in olfactory function. A significantly greater decrease in nasal airway caliber occurred following allergen challenge as compared to buffer diluent challenge in the same individuals (70% ± 7% versus 22% ± 5%). During an allergic response, a strong correlation was found between the minimum cross-sectional area and the volume of the nasal cavity measured by acoustic rhinometry ($r = .9$). However, no correlation was observed between nasal airway caliber and concomitant subjective congestion reported by the subjects. A modest decrease in olfactory function was seen following allergen challenge (3.1 ± 1.4 fewer odors identified correctly out of 20; $p = .08$). However, the alterations of olfactory function did not correlate with changes in nasal patency. The results presented in this study demonstrate that acoustic rhinometry has great potential as a reproducible method for the objective assessment of nasal obstruction occurring in nasal allergen challenge studies.

KEY WORDS — acoustic rhinometry, nasal allergy, nasal patency, nasal provocation.

INTRODUCTION

In the immediate nasal allergic response, the release of mast cell products and other inflammatory mediators leads rapidly to the cardinal symptoms of allergic rhinitis: pruritus, rhinorrhea, sneezing, and nasal obstruction. Nasal congestion is chiefly caused by mucosal inflammation, engorgement of venous capacitance vessels, and increased vascular permeability leading to mucosal edema. Histamine, leukotrienes, tumor necrosis factor–α, substance P, and calcitonin gene–related peptide have all been implicated in its pathogenesis.[1] Much research has been undertaken to study the mechanisms of nasal allergy, as well as to evaluate potential therapies. Nasal allergen challenge has been recognized to be a useful model for the examination of nasal allergic response physiology. A variety of techniques developed for the clinical assessment of nasal patency have been adapted for use in nasal provocation studies. The most commonly utilized method is anterior rhinomanometry, although it is somewhat cumbersome and has high coefficients of variation in repetitive measurements.[2,3] Rhinomanometry provides an indirect estimate of airway dimensions that often correlates poorly with an individual's subjective sense of congestion.[2,4] For the lack of a better, more reliable technique to measure airway dimensions, it has long been difficult to objectively assess the effect of allergen challenge on nasal patency.

Acoustic rhinometry is a recently described technique that makes use of reflected sound to estimate nasal geometry. Acoustic reflections from the nose are collected by a microphone and analyzed with an analog-to-digital converter by a computer. This information is processed to determine the minimum cross-sectional area (MCA) of the nasal cavity as a function of distance from the nasal aperture. It is noninvasive, rapid, and relatively simple to perform and requires minimal cooperation from the subject.[5,6] Since its first description by Hilberg et al[7] in 1989, acoustic rhinometry has repeatedly proven to be a reproducible and accurate technique for the assessment of nasal patency. The coefficients of correlation between measurements of MCA by acoustic rhinometry and measurements made by other methods such as computed tomography, rhinomanometry, and water

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displacement in baseline evaluations have been reported as .90 to .96.[8] Several recent studies have made use of acoustic rhinometry to measure changes in nasal patency associated with posture, the nasal cycle, localized skin cooling, allergic and vasomotor rhinitis, and surgical procedures.[9-19]

As compared to rhinomanometry, acoustic rhinometry is particularly well suited to the study of the immediate phase of nasal allergic reactions for at least three reasons. First, rhinomanometry measures nasal resistance, which is largely determined by the flow-limiting segment of the nasal airway, the nasal valve region. Airflow through this limiting anatomic segment depends in great part on the degree of congestion of the anterior end of the inferior turbinate. In an allergic response, there is widespread engorgement of the nasal mucosa, decreasing the dimensions of the nasal cavity throughout, including the nasal valve region. Acoustic rhinometry can examine changes in nasal airway caliber globally, while rhinomanometry indirectly reflects inflammation of only the inferior turbinate in the flow-limiting segment.

Second, in experimental models of allergic disease, testing of nasal patency often needs to be repeated many times over the course of an allergen challenge. Acoustic rhinometry has the advantage of being relatively simple and quick to perform.

Third, since acoustic rhinometry is noninvasive and does not require active breathing through the nose, it does not perturb the progression of a provoked allergic response.

In this pilot study we demonstrate the applicability of acoustic rhinometry in the investigation of nasal congestion in an allergen challenge model. In addition, we compare acoustic rhinometric results with subjective congestion and other measures of the allergic response.

METHODS

Eight subjects between the ages of 22 and 37 were recruited on the basis of a history of seasonal allergic rhinitis and a positive intradermal skin test to 10 or less protein nitrogen units (PNU) of grass pollen extract per milliliter. All subjects were symptom-free, not on any medications, and in good physical health at the time of the study. The protocol was approved by the University of Pennsylvania Institutional Review Board Committee on Studies Involving Human Beings, and each subject gave informed consent.

In the study, the response of subjects to intranasal challenge with antigen on one day was compared with the response to challenge with the diluent used in the antigen solution (phosphate-buffered saline; PBS) on a separate day, separated by at least 1 week. Subjects were randomly assigned to receive either antigen or buffer challenge on the first challenge day. On the first challenge day, one of the two nasal cavities was chosen at random for all spray challenges and measurements. The same nasal cavity was used on the second challenge day in that subject.

Each challenge day began with two baseline measurements of nasal patency 15 minutes apart in the designated nasal cavity. In between these measurements, the subject performed the University of Pennsylvania Smell Identification Test (UPSIT), in which the individual is asked to identify 20 distinct odors in a coded, standardized manner.[20] The score on each test is defined as the number of correctly identified odors.

Next, four sequential challenges were carried out in the designated nasal cavity by means of a metered-dose inhaler, which delivered a consistent volume of 0.3 mL. On the control challenge day, one spray of only the buffer solution was delivered on four occasions at 10-minute intervals. The antigen challenge day involved one spray of buffer followed by one spray of three increasing concentrations of grass antigen extract (10, 100, and 1,000 PNU/mL) at 10-minute intervals. Two minutes after each spray, a 7-mm filter paper disk was applied to the anterior portion of the nasal septal mucosa to collect nasal secretions. The disk was removed after being in place for 5 minutes and placed in a preweighed tube of PBS at 4°C. Following disk removal, the subjects graded their rhinorrhea, congestion, and itchiness on scales of 1 to 5 (with 5 being the most severe), and sneezes were counted. Acoustic rhinometric measurements were then performed 8 to 9 minutes after each challenge. Subjects were followed for 40 to 50 minutes after the fourth spray, with acoustic rhinometry repeated every 10 minutes. The UPSIT was performed 20 minutes after the final spray on both the antigen challenge and control challenge days. At the completion of the challenges and subsequent observation period, nasal scrapings of the middle turbinate were obtained with a Rhinoprobe (Apotex Scientific, Arlington, Tex), smeared on slides, and fixed in 95% ethanol for analysis of nasal cytology.

The filter paper disks were weighed following removal after each challenge and placed in the preweighed container of PBS to determine the weight of collected secretions, then incubated overnight at 4°C in the PBS. The container was centrifuged the following day and the supernates were frozen in aliquots at –70°C for later analysis of histamine content by enzyme-linked immunosorbent assay (Amhac Inc,

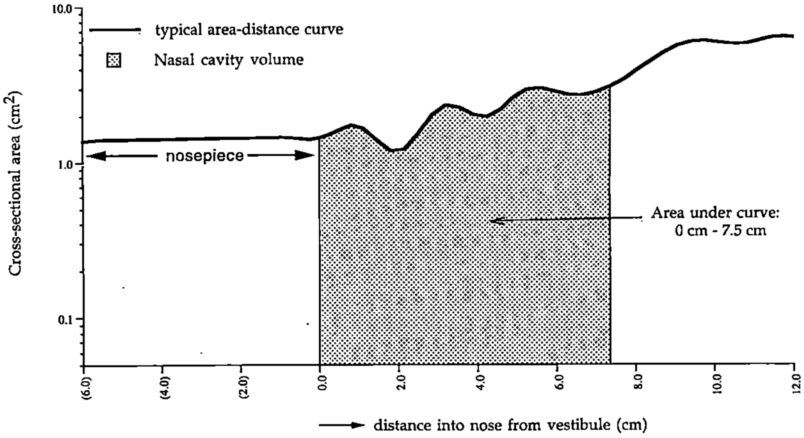

Fig 1. Typical cross-sectional area versus distance graph generated by acoustic rhinometry (solid line). Shaded region represents volume of nasal cavity over first 7.5 cm from vestibule.

Westbrook, Me).

Acoustic rhinometry was carried out with an acoustic rhinometer prototype similar to the model used by Dr Ole Pedersen of Denmark and Dr David Swift of Johns Hopkins University. This machine makes use of IBM computers with analog-to-digital converters for data acquisition and processing. Data were transferred to an Apple Macintosh computer for data analysis and graphic presentation. In this way, a cross-sectional area-distance curve for a distance of 12 cm from the nasal vestibule into the nasal cavity was described graphically by the apparatus (Fig 1). The MCA was defined at the lowest point of the curve. The nasal cavity volume was defined for this study as the area under the area-distance curve for the first 7.5 cm distance into the nose from the nasal vestibule. Any percentage change in nasal cavity volume or MCA after a challenge was defined as baseline (prechallenge) value minus the postchallenge value divided by the prechallenge value. The changes observed following allergen challenge versus buffer challenge were compared by means of paired *t*-tests. Paired *t*-tests were also used to compare peak histamine release following allergen versus buffer challenge. Correlations between acoustic rhinometric findings and other parameters were determined by Spearman rank-correlation analysis.

TABLE 1. FREQUENCY AND INTENSITY OF NASAL SYMPTOMS FOLLOWING NASAL ALLERGEN CHALLENGE IN SENSITIVE SUBJECTS

	Challenge Allergen Concentration			
Symptom	*10 PNU/mL*	*100 PNU/mL*	*1,000 PNU/mL*	*15 Minutes Postchallenge*
Rhinorrhea	5 (1.6)	8 (2.1)	8 (4.1)	8 (2.5)
Itchiness	5 (1.2)	6 (1.3)	7 (2.1)	4 (1.8)
Congestion	2 (2)	5 (2.6)	8 (3.4)	8 (3.3)
Sneezing	0	3 (1)	6 (5)	0

Data are numbers of subjects exhibiting symptom out of eight subjects studied. Values in parentheses are mean symptom intensity scores for eight subjects on 1 to 5 scale, with 5 being most intense.

PNU — protein nitrogen units.

RESULTS

Symptomatic allergic responses were induced in all eight subjects after challenge with the three graded concentrations of grass pollen extract. Subjects complained of nasal congestion of at least 3 on a scale of 1 to 5 at the height of their symptomatic response. Variable degrees of sneezing, rhinorrhea, and itchiness were observed over the challenge period (Table 1).

In seven of the eight subjects, nasal allergen challenge induced a significant reduction in both nasal volume and MCA, as detected by acoustic rhinometry. For the whole group of eight subjects, the greatest decreases from baseline values following allergen challenge were 58% ± 5% (range 46% to 74%) for nasal volume, and 70% ± 7% (range 51% to 91%) for MCA. A very strong correlation between the absolute values for nasal volume and MCA was seen in individual measurements ($r = .9$). A representative cross-sectional area-versus-distance curve for an allergen challenge is shown in Fig 2.

On the control challenge day, none of the subjects reported allergic symptoms during the buffer challenges. The maximum decreases were 17% ± 4% (range 11% to 37%) for nasal volume and 22% ± 5% (range 0% to 37%) for MCA. Figure 3 shows the relationship between the range of area-distance curves seen during repeated buffer challenges and the area-distance curves following allergen challenge in the same subject.

The findings after antigen and buffer challenges in individual subjects were considered in another way. Table 2 compares the maximal percent changes in MCA and nasal cavity volume for each subject during allergen challenges and buffer challenges. A significant correlation was observed in this study

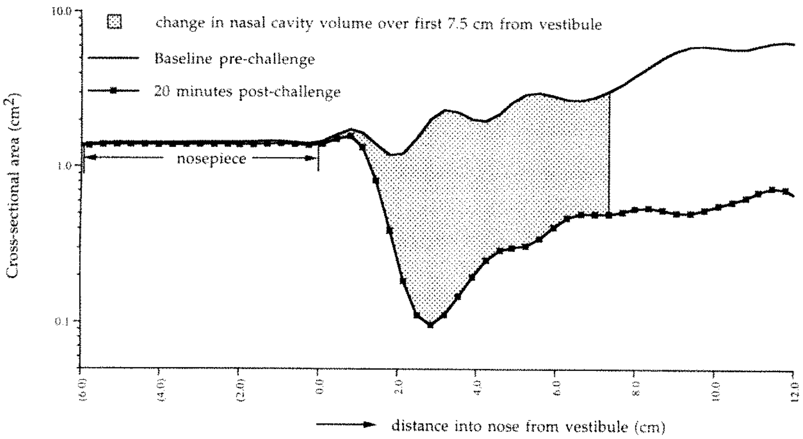

Fig 2. Decrease in nasal cavity volume following allergen challenge. Solid line represents baseline cross-sectional area-versus-distance function obtained prior to allergen challenge. Dotted curve shows area-distance function seen 20 minutes after challenge with 1,000–protein nitrogen unit (PNU) concentration of grass pollen in grass-sensitive individual. Shaded region is decrease in nasal cavity volume over first 7.5 cm from nasal vestibule.

group between the greatest subjective congestion and the maximal decrease in MCA following allergen challenge (r = .8), by Spearman rank-order analysis. Thus, the greatest relative decrease in MCA was observed in those subjects who described the greatest subjective congestion. However, the greatest decrease in MCA and the most prominent subjective congestion did not necessarily occur at the same point in time during allergen challenge in individual subjects. Thus, no significant correlation was found between nasal patency and *concomitant* subjective congestion. A comparison of sequential nasal volume changes and subjective congestion scores in an individual subject can be seen in Fig 4.

Olfactory measurements with a standardized panel of 20 different odors were made before and after challenge with allergen and buffer diluent, and expressed as the difference in the number of correctly identified odors before and after challenge. For the group of eight subjects, there was essentially no difference in odor perception following the buffer challenges (−0.625 ± 0.46). A modest decrease in the number of correctly identified odors occurred during the allergen challenges (−3.12 ± 1.4; $p = .08$, paired *t*-test). Differences in olfactory function following allergen challenge were not correlated quantitatively with objectively measured changes in nasal patency.

Changes in nasal patency were also not correlated with the degree of subjective rhinorrhea or pruritus. There was a significantly greater peak increase in the volume of nasal secretions following allergen challenge than after buffer challenge (35 ± 6.2 versus 2.5 ± 1.6 mg; $p < .001$), which also did not correlate with changes in nasal patency. However, there was a suggestion of an inverse relationship between nasal patency and the number of sneezes occurring after allergen challenge.

There was a significant increase in the peak histamine content of the nasal secretions following the allergen challenges as compared to that following the buffer challenges (6.0 ± 1.6 versus 1.0 ± 0.34; $p = .01$). However, no correlation was observed between the amount of histamine eluted from the secretions and decreases in nasal patency as assessed by either acoustic rhinometry or subjective congestion. In two of the eight subjects, the maximal histamine levels were found in secretions obtained after the 10-PNU challenge, while in three of the eight subjects the maximal histamine levels were seen after both the 100-PNU and 1,000-PNU sprays.

Fig 3. Decrease in airway caliber in response to allergen challenge as compared to buffer challenge. Shaded region represents range of cross-sectional area-versus-distance curves generated over buffer challenge day. Solid line shows baseline area-distance curve from allergen challenge day prior to allergen challenge. Note that it falls largely within shaded region. Other curves depict area-distance functions following 100-PNU challenge and at two time points following 1,000-PNU challenge.

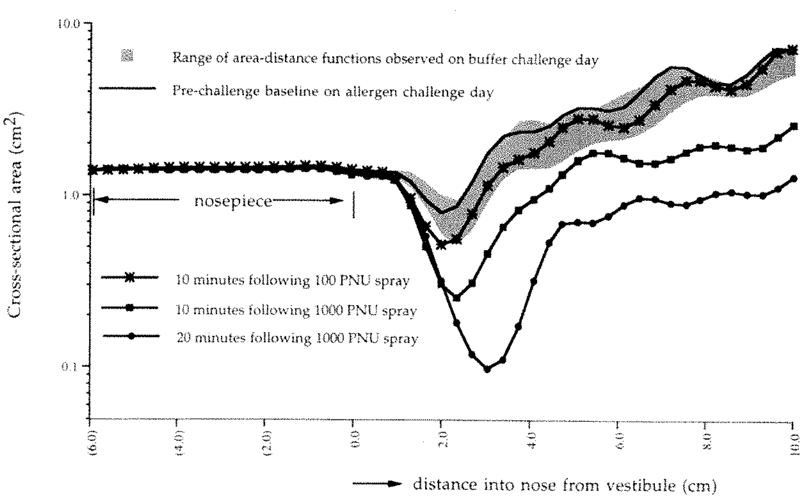

TABLE 2. CHANGES IN NASAL CAVITY VOLUME, MINIMAL CROSS-SECTIONAL AREA, AND SUBJECTIVE CONGESTION FOLLOWING NASAL CHALLENGE

	Antigen Challenge		*Buffer Challenge*		*Maximum Subjective Congestion§*
Subject	*Decrease in Volume**	*Decrease in MCA*	*Decrease in Volume†*	*Decrease in MCA*	
1	58%	68%	27%	21%	5
2	58%	72%	21%	22%	4
3	46%	51%	6%	28%	3
4	31%	32%	26%	37%	3
5	59%	82%	3%	0%	4
6	74%	91%	4%	0%	5
7	73%	85%	27%	34%	4
8	67%	83%	26%	32%	4

MCA — minimal cross-sectional area.

*Greatest decrease in nasal cavity volume (as compared to baseline).

†Decrease in nasal cavity volume during buffer challenge at time following baseline corresponding to time when maximal decrease in nasal cavity occurred during allergen challenge in same subject.

§On scale of 1 to 5, with 5 being most severe.

Following allergen challenge, prominent numbers of inflammatory cells (mainly neutrophils) were seen in nasal mucosal scrapings of most subjects. However, the extent of this response did not correlate with nasal patency measurements. In two of the eight subjects, large numbers of eosinophils were observed in the nasal scrapings. In the remaining subjects, only occasional eosinophils were seen. However, the decrease in nasal airway caliber was not the most prominent in the two subjects with pronounced nasal eosinophilia.

DISCUSSION

Nasal congestion is reported commonly by individuals undergoing experimentally induced acute nasal allergic reactions. However, there has been a long-standing unmet need for a satisfactory repetitive, easily performed measurement of nasal patency.[21] The vast majority of previous attempts to objectively assess changes in airway caliber thought to be responsible for this subjective sensation of congestion have utilized rhinomanometry.[2,3,21] However, this technique measures the resistance to nasal airflow, rather than directly measuring the airway caliber or nasal volume. Since nasal resistance is largely determined by the flow-limiting segment, or the region of the airway with the smallest cross-sectional area, rhinomanometry is a functional measurement that indirectly describes nasal patency over a limited anatomic region only.

This fact may explain the lack of good correlation observed between the subjective sensation of congestion and rhinomanometric changes in allergic reactions.[4] Also, paradoxically, subjective nasal congestion is commonly reported by those with atrophic rhinitis, even though nasal airway resistance in these individuals measured by rhinomanometry is very low.[4] On the other hand, inhalation of certain vapors (eg, eucalyptus, menthol) is often followed by a sensation of decreased nasal congestion even though

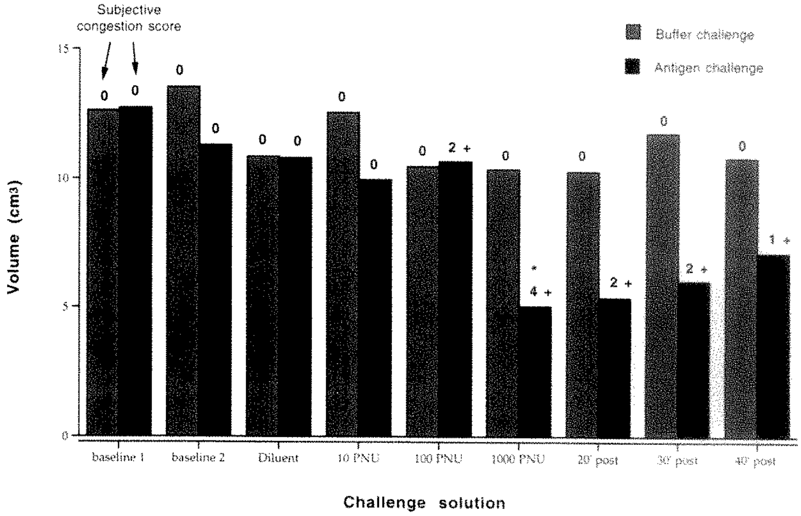

Fig 4. Nasal volumes and subjective congestion score in representative individual. Lighter-shaded bars represent nasal cavity volume at different time points on buffer challenge day. Darker bars show nasal cavity volumes at corresponding time points on allergen challenge day. Subjective congestion score reported by individual at each time point is displayed above each bar. Maximum objective congestion determined by acoustic rhinometry (58% from baseline) coincided with maximum subjective congestion (4+ out of 5) following 1,000-PNU spray. Decrease in volume at corresponding time point during buffer challenge day was 21% from baseline.

no significant changes in airway resistance occur.[22] These findings suggest that subjective nasal congestion is not simply a reflection of increased airflow resistance at the nasal valve. Therefore, a more direct assessment of nasal airway caliber and nasal cavity volume during acute allergic reactions in the nose is desirable.

In this study, we have demonstrated the utility of acoustic rhinometry in measuring the sequential, dynamic changes occurring simultaneously in nasal airway caliber and nasal cavity volume during the acute allergic response. We have found the technique to be relatively simple and rapidly performed, with very little variation in repeated measurements. Since it is noninvasive and does not require nasal airflow, acoustic rhinometry during the challenge does not appear to perturb the evolution of the provoked nasal allergy.

The nasal mucosal responses following allergen challenge observed in this study were manifested by prominent decreases in both nasal cavity volume and in MCA as determined by acoustic rhinometry. Decreases in nasal cavity volume, which was measured over a distance of 7.5 cm beginning at the nasal vestibule, are a reflection of global airway narrowing. On the other hand, MCA generally occurred in the nasal valve region, primarily determined by inflammation of the head of the inferior turbinate. Nasal cavity volume and MCA measurements were very strongly correlated in individual measurements, demonstrating that changes in nasal patency during acute allergic reactions occur rather uniformly, at least over the first 7.5 cm into the nose. Although our distance-area curve findings beyond the first 7.5 cm suggest a further marked decrease in airway caliber in that part of the nose, the precision of acoustic rhinometry may be uncertain in the posterior aspects of the nose, particularly beyond 12 cm from the nasal opening.[8]

In the study group reported here, the greatest relative decreases in nasal caliber and volume were seen in those subjects reporting the most subjective congestion at some time during the challenge. However, there was no consistent temporal relationship between the onset of such congestion and the first observed clear-cut decrease in airway caliber. This lack of temporal correlation may not be surprising, since we found the MCA most commonly in the nasal valve region, possibly at a distance from the neural network that conveys a sense of nasal congestion. However, we also found that the degree of subjective congestion did not correlate temporally with decreases in nasal cavity volume, a more global assessment of airway patency. Indeed, maximal subjective congestion preceded the greatest decrease in nasal volume in five of the eight individuals by as long as 40 minutes. The modest decreases in nasal cavity volume (17%) and MCA (22%) following buffer challenge may reflect normal nasal cycling and/or nonspecific effects of the spraying process, including possible effects of the preservative present in the solutions.

As expected, there were other symptomatic and inflammatory responses in these subjects observed during allergen challenge but not control buffer challenge. Prominent sneezing occurred in six of the eight subjects during challenge with the highest allergen concentration; however, if anything, less decrease in airway caliber was seen in those who sneezed the most. The weight of nasal secretions (encompassing both the volume and density of secretions) also did not correlate with the decrease in airway caliber at individual time points. We found inflammatory cell infiltrates present in all subjects 45 minutes after the final allergen challenge, the extent of which did not correlate with nasal congestion. As observed previously by others looking at the acute allergic response in the nose,[23] the infiltrate was predominantly composed of neutrophils, with a variable number of eosinophils, perhaps corresponding to their proportion in the circulating blood. On the basis of previous nasal challenge studies, one would expect a more prominent eosinophil exudate in scrapings obtained hours after allergen challenge.[23] The amount of histamine released locally onto filter paper disks during allergen challenge was significantly greater than that following buffer challenge in all subjects — similar to the findings of other groups using nasal allergen challenge.[24] One would expect the amount of histamine released to correlate with subjective congestion and/or decreases in airway patency, since histamine sprayed into the nose induces prominent nasal congestion.[25] However, in our study, peak histamine levels were seen at various time points after allergen challenge in different individuals and did not correlate with other parameters measured.

It has been reported that over 50% of patients with allergic rhinitis have decreased olfaction at one time or another.[26] It has been assumed that this decreased sense of smell is associated with nasal obstruction. However, in both normals and allergic rhinitis patients, no correlation has been found between diminished sense of smell and decreased nasal patency, as assessed indirectly by rhinomanometry.[26]

The findings reported here are the first, to our knowledge, of altered olfactory function during nasal allergen challenges, and the first comparison of such olfactory alterations with changes in nasal patency assessed by acoustic rhinometry. Although we found modest decreases in olfaction after the induced allergic reaction (p = .08), these changes did not correlate with the degree of reduction in nasal patency. This lack of correlation is similar to that seen in rhinomanometric studies during naturally occurring allergic rhinitis. Clearly, hyposmia in nasal congestion is due to more than nasal congestion, even when the latter is evaluated by the more global assessment of nasal cavity volume. It may be that hyposmia in allergy is due to local inflammatory effects, with or without obstruction, in the olfactory region of the nasal cavity that cannot be detected with sufficient sensitivity by either rhinomanometry or acoustic rhinometry.

In summary, the findings reported here indicate that nasal patency, as defined by two well-correlated indices, can be measured readily by acoustic rhinometry on repeated occasions during experimentally induced acute allergic reactions. Significant diminution in airway caliber and nasal cavity volume occurs in almost all subjects, with the greatest decreases observed in those describing the most subjective congestion. However, there is no precise temporal correlation between these two events, nor with the modest degree of hyposmia induced and the extent of histamine release or inflammatory cell exudation. In experimental models of allergy designed to elucidate the physiologic mechanisms of the allergic response or those designed to evaluate potential therapeutic interventions, it is important to have standardized, objective methods for assessing changes in nasal patency and its modulation by pharmacologic therapies. Acoustic rhinometry may prove to be an invaluable technique in the future for the objective assessment of nasal congestion in allergen challenge models of allergic disease.

ACKNOWLEDGMENT — We thank Jana Kesavanthan at Johns Hopkins University School of Hygiene and Public Health for her kind assistance in the modification and maintenance of the acoustic rhinometry apparatus used in this study.

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The Twenty-Fourth Ordinary Congress of the International Neurootological and Equilibriometric Society Reg. (NES) will be held April 6-10, 1997, in Haifa, Israel. For information, contact NES Congress President Prof Dr Ludwig Podoshin, Otolaryngology, Head and Neck Surgery, Bnai Zion Medical Center, PO Box 4940, Haifa 31048, Israel; telephone 972-4-359544; fax 972-4-371393.

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Ann Otol Rhinol Laryngol 105:1996

PHARYNGEAL DERMOIDS ("HAIRY POLYPS") AS ACCESSORY AURICLES

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The purpose of this study is to clarify the origin and nature of so-called hairy polyps or dermoids of the pharynx, which are often thought to be a variant of pharyngeal teratoma. For this purpose, a case is reported of a dermoid polyp involving the middle ear of an infant, the features of multiple examples of pharyngeal dermoid polyps and teratomas received for consultation by the Armed Forces Institute of Pathology are examined, and selected pertinent reports from the literature are reviewed. All three means are used to support the conclusion that these lesions are choristomatous developmental anomalies arising from the first branchial cleft area and that they essentially represent heterotopic accessory "ears" (auricles) without the growth potential of a teratoma.

KEY WORDS — accessory auricle, choristoma, dermoid; middle ear, pharynx, teratoma.

INTRODUCTION

Arnold[1] in 1888 described four types of congenital pharyngeal malformations: 1) epignathus, with grossly very well-developed anatomic structures, 2) pharyngeal teratoma, 3) pharyngeal "teratoid," with poorly differentiated structures or tissues, and 4) pharyngeal dermoid. Category 1 is the type of abnormality that would suggest a maldeveloped twin, or fetus in fetu, as the genesis of the malformation. Category 2 would seem to correspond to what might be called a conventional teratoma, or the type usually encountered in various anatomic locations. Category 3 is one that is difficult to clearly delineate within more modern ideas of pathologic classification. Category 4 is the subject of this present study.

Schwalbe[2] in 1907 had a slightly different list of four categories, all considered subcategories of epignathi: 1) pharyngeal masses with remarkably well-differentiated structures, including umbilical cord formation (these cases must be "vanishingly" rare), 2) masses with grossly well-differentiated organoid structures, 3) "conventional" teratoma, and 4) dermoids.

While Arnold[1] apparently did not explicitly denote the pharyngeal dermoid as a variant of teratoma, some subsequent classifications (eg, especially, Schwalbe's[2]) implied that the pharyngeal dermoids are within the spectrum of teratoma; ie, they could be construed as a limited form of teratoma. Some authors in recent decades have not labeled the pharyngeal dermoids as teratomas, but rather, have considered them to be choristomas.[3] However, there are authors who still imply that hairy polyps are a limited teratoma.[4]

Although admittedly not a profound clinical question, determining that these malformations are not teratomas (and therefore do not have the growth potential of a teratoma and certainly no malignant potential, in contrast to some teratomas) is clinically relevant. The evidence presented herein that supports a specific developmental provenance for pharyngeal dermoid polyps is probably of some academic interest for furthering the detailed knowledge of these specific developmental anomalies.

CASE REPORT

A 16-month-old girl was evaluated at an otolaryngology clinic in the interior of Alaska for recurrent otitis media and right otorrhea. She had a history of recurrent otitis media (mostly on the right side) since age 7 months (>12 episodes). Her language development had been appropriate for age. She had no prior surgery or hospitalizations and was on no medication. The examiners noted a soft tissue mass filling the medial half of the external ear canal. An outpatient clinic biopsy revealed keratin, suggestive of a cholesteatoma. A computed tomography (CT) scan demonstrated a mass filling the right external auditory canal and middle ear, extending into the mastoid, hypotympanum, protympanum, and pterygomaxillary

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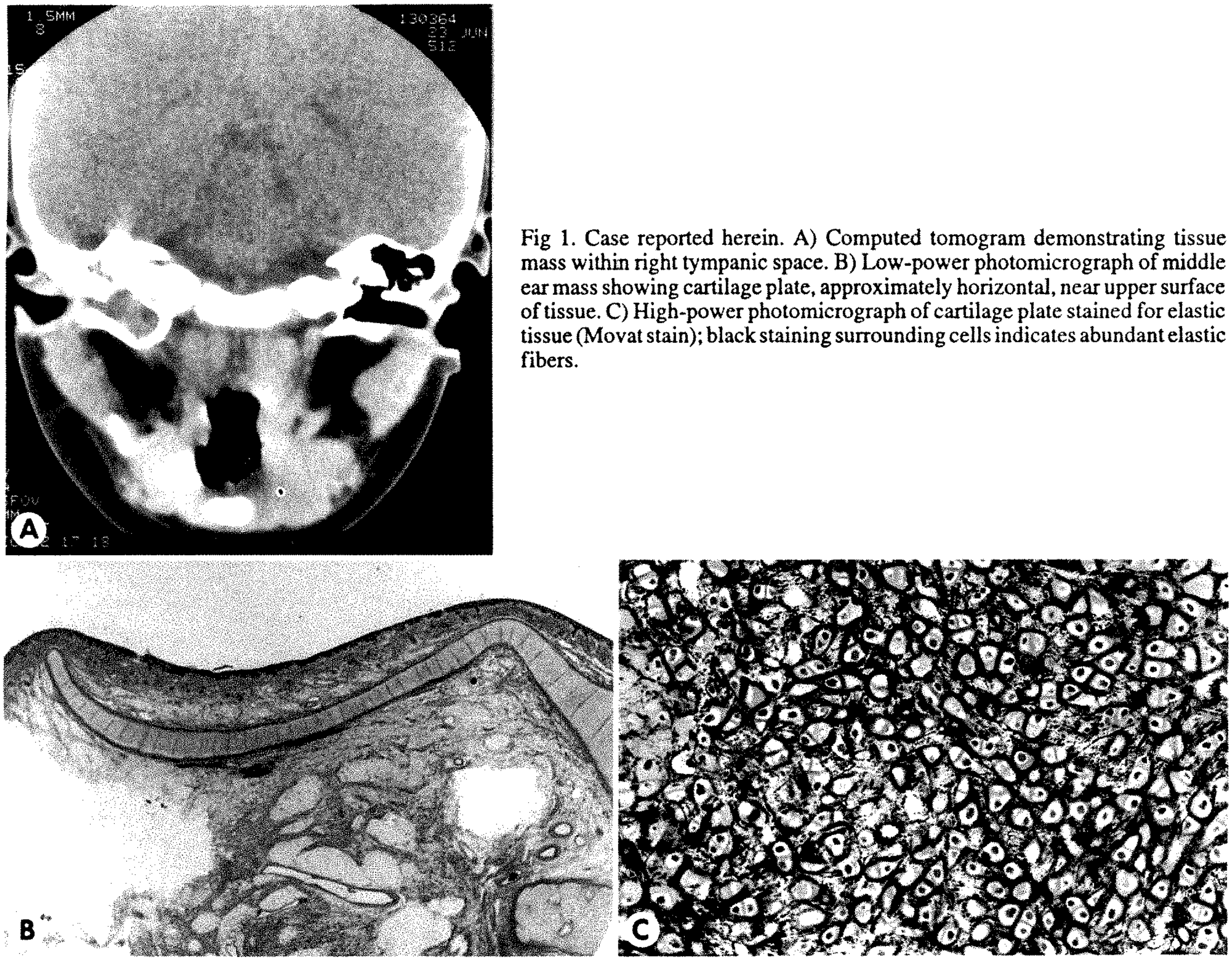

Fig 1. Case reported herein. A) Computed tomogram demonstrating tissue mass within right tympanic space. B) Low-power photomicrograph of middle ear mass showing cartilage plate, approximately horizontal, near upper surface of tissue. C) High-power photomicrograph of cartilage plate stained for elastic tissue (Movat stain); black staining surrounding cells indicates abundant elastic fibers.

space, and which apparently exposed the carotid artery. There appeared to be dehiscence of the middle fossa dura. The patient was then transferred to Madigan Army Medical Center (MAMC) for further evaluation.

Examination at MAMC confirmed the above findings. Facial nerve function was normal. Cranial nerves II through XII were intact except for cranial nerve VIII on the right. There was no nystagmus noted. Audiometric testing revealed speech awareness thresholds at 20 dB hearing level for the better-hearing ear. Immittance was normal in the left ear and abnormal on the right. Additional CT views (Fig 1A) confirmed the previously noted findings, except that the carotid artery appeared to be covered.

The patient was taken to the operating room, where multiple biopsies of the external ear canal mass were performed. Frozen section results revealed keratin material without evidence of rhabdomyosarcoma. The soft tissue mass was then debulked to the level of the tympanic membrane. No tympanic membrane was found; however, a bar of horizontally situated cartilage similar to tragal cartilage was noted at the level of the annulus. A mastoidectomy was then performed. Squamous debris was encountered at the level of Koerner's septum, filled the medial mastoid, extended into the middle ear, and abutted the mass. At this point, because of the extensive squamous debris, a canal wall down procedure was undertaken. The incus and malleus were noted to be displaced superiorly by the mass. The mass was then mobilized and it seemed to be attached to (and perhaps arising from) the area of the tympanic annulus; it was grasped and rotated out of the middle ear, hypotympanum, and pterygomaxillary space. The carotid artery was covered and the stapes superstructure was intact except for the capitulum. No dural dehiscence was noted. There was no aberrancy of the facial nerve. A meatoplasty was performed and the procedure terminated.

Pathologic examination revealed that the lesion was not a cyst, but rather, was a solid polypoid structure. On the histologic slides, the specimen contained a well-formed cartilage plate that was thin and elongated, measuring approximately 1.0 mm in width and approximately 18 mm in extent (Fig 1B).

The cartilage was elastic cartilage (manifesting elastic fibers on an elastic tissue stain) and very much resembled auricular cartilage (Fig 1C). Surrounding the cartilage plate was adipose tissue similar to subcutaneous tissue, and surrounding this and lining the surface of the specimen was skin (dermis and epidermis with adnexal structures, including hair appendages). The histologic similarity to auricular tissue was clearly apparent — especially the conformation of the cartilage.

The patient has done well for 12 months postoperatively without complications.

ADDITIONAL MATERIALS AND METHODS

Eight cases of pharyngeal dermoid polyps and four cases of pharyngeal teratomas retrieved from the Armed Forces Institute of Pathology (AFIP) consultation files were studied. The histologic material was scrutinized for the details of tissue composition, especially the exact structure of the cartilage, including the size and general conformational outline and whether or not there was evidence of elastic cartilage. The AFIP material was also used to compare the tissues of pharyngeal teratomas with those of hairy polyps, with particular attention once again given to cartilage. Also, comparison of the histologic features of the hairy polyps was made to the features of developing fetal auricles furnished by one of the authors (V.A.).

Literature reports of hairy polyps were scrutinized for the location of origin of the lesions, the gross appearance, associated malformations, and the details of tissue composition. In the last instance, the exact structure of the cartilage was considered apposite, including the size and general conformational outline and whether or not there was evidence of elastic cartilage.

RESULTS

The literature review indicated that our hypothesis that pharyngeal dermoids are accessory auricles is not exactly new. A few reports[5,6] described hairy polyps that have a gross resemblance to (malformed) auricles. The logical inference was made in these reports that the hairy polyps were probably of first or second branchial arch origin, and one report[6] explicitly labeled the pharyngeal lesion as an accessory auricle. However, these reports (including the latter) did not explicitly or fully develop the argument that pharyngeal hairy polyps in general are accessory auricles. Moreover, these reports are few, and the idea that pharyngeal hairy polyps might be accessory auricles is certainly not widely known.

In the literature, the term dermoid is often considered synonymous with dermoid cyst. This is reasonable, since dermoid lesions in most parts of the body (including the older term of dermoid cyst of the ovary, which is now recognized to more accurately be a benign cystic teratoma) are indeed cysts. It seems to be tacitly assumed by some that all dermoid lesions are cysts. This can be seen in reports of dermoids that are entitled with "dermoid cyst," and yet the description of the lesion does not seem to indicate a cystic lesion.[7,8] It is clear from the literature that pharyngeal dermoids actually are not dermoid cysts, but rather, are solid polypoid lesions.

Although pharyngeal dermoids can arise from the soft palate (nasopharyngeal surface) or in the oropharynx, including the tonsillar region, a majority of these malformations occur laterally in the vault of the nasopharynx and therefore are not far from a eustachian tube orifice. Some even appear to be arising within the eustachian tube and protruding from it.[8,9] There are reports of dermoids entirely confined to the eustachian tube.[10]

There are also a few reports of dermoids of the middle ear or mastoid area.[7,11-13] It is often difficult to tell from the reported information whether the lesion was solid or truly cystic. At least one may have been a small cyst,[13] but others were more likely solid polypoid lesions.[7] In at least one instance the lesion appeared to arise from the eustachian tube and extended into the tympanic cavity.[11]

The very large majority of pharyngeal, eustachian tube, or tympanic dermoid polyps occur in patients who have normal auricles. However, there are a few reports of patients with pharyngeal dermoid polyps and associated malformations of the auricle.[12,14]

Since pathologic material was available in the AFIP cases, it could be examined in more detail (including elastic tissue stains on cartilage) than is generally available in literature reports, which often do not have histologic pictures or information regarding the type of cartilage. Examination of our cases of dermoid polyps revealed most to have prominent cartilage within the mesenchymal tissues. Usually the cartilage was in the form of an elongated or curved plate of relatively uniform thickness and was approximately as thick as normal auricular cartilage (Fig 2). Elastic cartilage was not well developed in most specimens, but in two cases it was extremely well developed, with striking elastic fibers manifested with an elastic fiber stain. The strong resemblance to auricular cartilage was most impressive. For the eight cases examined, the degree of elastic cartilage differentiation could not be significantly correlated with the age of the patient. Comparison of

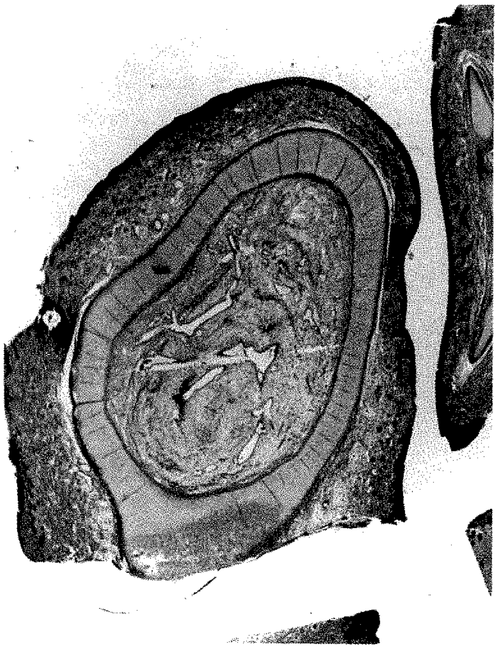

Fig 2. Low-power photomicrograph of Armed Forces Institute of Pathology case of "pharyngeal hairy polyp" (ie, accessory auricle) with section through circular plate of cartilage with thickness similar to that of normal auricular cartilage.

the histologic appearance of the dermoid polyps to the appearance of fetal auricles confirmed the general resemblance of the polyps to auricles.

Although the cases of congenital nasopharyngeal teratomas contained cartilage, extensive well-formed plates of uniform thickness resembling auricular cartilage were not seen. The cartilage was generally more cellular and immature in appearance than was the cartilage found in the dermoid polyps. There was one small focus in one case that manifested elastic fibers, but the other, larger areas of cartilage in this case did not.

DISCUSSION

The hypothesis that pharyngeal dermoid (hairy) polyps are actually accessory auricles was generated by pondering a few simple observations. The term dermoid generally connotes a dermoid cyst, which usually is thought to probably develop from an inclusion, or entrapment, of ectodermal tissue with the capacity to form skin.[15,16] With respect to the pharyngeal dermoids, this concept presents two main difficulties, both of which serve as clues that these lesions are something a bit different from the usual dermoid. First, they are solid, exophytic masses, and second, they are not close to a cutaneous site. The solid, exophytic growth does not suggest a developmental inclusion or entrapment phenomenon. Their not being close to a cutaneous site presents a significant problem for explaining the embryogenesis of these polyps, although it has not seemed to be a problem to other authors.[17] Since these lesions are not far from the ectodermally derived mucosa of the mouth or nasal cavity, other authors have related these areas to the development of dermoid polyps. However, lesions similar to pharyngeal dermoid polyps do not seem to occur in the sinonasal or oral mucosa. Dermoid cysts of the nasal bridge (aside from being cysts and not solid polyps) are related to and derived from a cutaneous area, not the schneiderian mucosa. Dermoid cysts or polyps of the nasal cavity proper or the paranasal sinuses are virtually unknown. Although dermoid cysts of the floor of the mouth are well known, these lesions possibly could be derived from midline upper neck or submental cutaneous areas rather than from stomodeal ectoderm. In any case, they are cysts rather than exophytic polyps. Most persons have tiny nests of sebaceous glands (Fordyce granules) in the oral cavity, and these structures are derived from oral cavity mucosa. However, they do not form gross polypoid masses.

If pharyngeal dermoid polyps are ectopically located malformations derived from sinonasal or stomodeal ectoderm, it seems quite unusual that similar lesions do not seem to form "nonectopically" in the sinonasal or stomodeal areas. This is unusual enough, in our thinking, that we strongly doubt the origins of these lesions postulated in the literature as possibly being from inclusions between palatal shelves[17] or remnants of the breakdown of the buccopharyngeal membrane.[18] If they are not from these areas (and if not teratomas), what would be the embryologic provenance of these lesions?

There is one area, other than the mouth or nasal cavity, in which the endodermal upper pharyngeal tissue is developmentally close to ectodermal tissue — indeed, very close. The first pharyngeal branchial pouch endoderm is usually said to contact the first branchial groove ectoderm (in the area that will ultimately develop into the tympanic membrane), and subsequently the two layers "pull away" as some mesodermal tissues develop between them. This seems the most plausible area for displacement of ectodermal tissue that subsequently becomes associated with the tympanum, eustachian tube, or pharynx. We believe this is indeed the origin of pharyngeal dermoid polyps, because of the additional evidence that indicates an auricular quality for the tissues of the dermoid polyps, and since, of course, the normal auricle develops around the first branchial cleft (from the ectoderm of the cleft and the adjacent first and second arch mesoderm).

Additional evidence is derived from the spectrum of exact locations of dermoid polyps. Most are in the nasopharynx and located laterally. This is not far from the eustachian tube. Some arise from the eustachian tube and project into the nasopharynx.[8] Eustachian tube origin may be more common than is reported, because the origin from the tube orifice may not be explicitly determined clinically in some cases.[8] Rare examples have been confined to within the tube proper.[10] Finally, there are instances of dermoid polyps within the middle ear space (as reported herein), some of which appear to be arising from the tube.[12] This spectrum of locations for dermoid polyps strongly suggests an association with the first pharyngeal pouch, from which the eustachian tube develops.

What about the significant number of pharyngeal dermoid polyps that appear to arise from the nasopharyngeal side of the soft palate or from the faucial tonsillar area and thus are not next to the eustachian tube orifice? Does this argue against a first pharyngeal pouch relationship? Not significantly, since these areas are still related to the endodermal pharyngeal branchial apparatus. A dermoid polyp in the tonsillar area seems hardly significantly "ectopic" from the first pouch area, when it is recalled that the caudal border of the first pouch (and cleft) is the second arch and that the tonsillar area is related to the second arch. Dermoid polyps of the tonsillar area are discussed in the literature as probably of second branchial cleft origin. It would be more accurate to include the second *arch* in this proposed origin, since these solid polyps surely include arch mesoderm in their formation, and the second arch is also involved (in part) in the formation of the auricle. It is true that tonsillar dermoid polyps would seem to be more related to the second branchial apparatus than to the first, but this probably is not sufficient reason to separate them as something fundamentally different from the nasopharyngeal dermoids. After all, external cutaneous accessory auricles (auricular tags) can be located well down on the neck, significantly away from the auricle and perhaps more in a second cleft area, and yet no one suggests considering them a different type of lesion from a histologically identical one located closer to the auricle. Tonsillar dermoid polyps are histologically identical to external auricular tags, and they are probably related lesions.

Important support for the hypothesis that pharyngeal dermoid polyps are accessory auricles is provided by the characteristics of the cartilage found in many of them. The first important point has already been documented in several reports,[5,6] and this is the fact that some of these lesions grossly resemble malformed auricles. This resemblance includes an extended plate of cartilage of uniform thickness that grossly very much resembles auricular cartilage. However, another important aspect of this cartilage has not been discussed in the literature. Some of these lesions have strikingly elastic cartilage, as did the tympanic dermoid polyp reported herein (Fig 1C) and one of our pharyngeal cases. The cartilage in one of the cases reported by Chaudry et al[14] (their Figure 6) is similar, and one can discern elastic fibers from the illustration even without the benefit of color or a special elastic tissue stain. The reason this is important is that well-developed elastic cartilage is not found in many areas of the body. Aside from the external ear and eustachian tube, the only other areas with appreciable elastic cartilage are the epiglottis and portions of the nasal alae. Thus, the presence of well-developed elastic fibers in some cases of pharyngeal dermoid polyps makes the cartilage identical in every respect to auricular cartilage and very strongly supports the developmental relationship to the ear. This striking finding is probably the most important factor that indicates that these developmental anomalies are choristomatous accessory auricles.

The striking and extensive elastic tissue in some of these lesions is also an argument against their representing meager teratomas. Since elastic cartilage is so specialized and sparse in the body, one would not expect to find much elastic cartilage in teratomas in general, and indeed, this is the case. There was one small focus with elastic fibers in one of the AFIP nasopharyngeal teratomas, but in most teratomas this is not a finding. Also, most of the cartilage in the AFIP nasopharyngeal teratomas was more cellular and more immature in appearance compared to the cartilage in the dermoid polyps, and the overall appearance of the cartilage in these two types of lesions was quite different.

After having had the opportunity to see many unusual examples of head and neck disease over a period of 16 years, the senior author (D.K.H.) believes that classifications of pathologic conditions are almost certain to fail to easily classify or explain all the conditions one will encounter. The multitudinous variations of the manifestations of disease do not always fit into the categorical drawers constructed by the pathologist. This is particularly true of the wondrous spectrum of biologic variability manifested in developmental abnormalities.[19] Nature does not always entirely cooperate with the dividing lines of our diagnostic categories and terms. Thus, we do not claim to be able to obviously and clearly explain by the hypothesis of this paper every example of a pharyngeal lesion containing skin. It is possible that rare examples of pharyngeal dermoids are meager teratomas. For example, the case described by Aughton et al[20] seems to mostly fit the category of dermoid (hairy) polyp, but it was said to contain a small amount of primitive neural and mesenchymal tissue. We do claim, however, that the features of almost all pharyngeal dermoid polyps as a group constitute a type of lesion that is different enough from the usual teratoma in tissue composition and probable developmental genesis that it should be categorized as different from a teratoma. If the term teratoma is to maximally retain its useful connotations, it should not become overly broad in scope. Accordingly, an accessory auricle (or auricular tag) on the skin of the neck is not classified as a teratoma. Certainly, accessory auricles on the neck do not contain structures from all three embryonic germ layers (the endoderm is missing), nor do they have as much tissue heterogeneity as do most teratomas. Except for not being located on a cutaneous site, we believe pharyngeal dermoid polyps are essentially the same type of developmental anomaly as cervical accessory auricles.

Although rare teratomas of the temporal bone area exist,[21] it is likely that many of the lesions of the middle ear or eustachian tube that have been reported as teratomas are actually accessory auricles (dermoid polyps). On the other hand, some middle ear dermoids seem to actually be dermoid cysts[13] and in the strictest sense might not be accessory auricles. The external ear canal develops from the first branchial cleft, and developmental anomalies that recapitulate this process can form subcutaneous dermoid cysts and sinuses. Occasionally, one of these might form in such a way as to present mainly as a middle ear lesion. But the solid and polypoid dermoid lesions of the middle ear, eustachian tube, and pharynx develop, we believe, from embryological displacements of first and second arch tissue with "auricular hillock" potential (from which the auricle develops), and these lesions are appropriately considered to be, and should be labeled as, accessory auricles.

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Ann Otol Rhinol Laryngol 105:1996

SIMILAR DISTRIBUTION OF MAST CELLS AND SUBSTANCE P– AND CALCITONIN GENE–RELATED PEPTIDE–IMMUNOREACTIVE NERVE FIBERS IN THE ADULT HUMAN LARYNX

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The mechanisms causing supraglottic and subglottic edema in the human larynx are not fully understood. Substance P (SP)– and calcitonin gene–related peptide (CGRP)–containing nerve fibers and mast cells have been suggested to induce inflammation and edema in other parts of the body. In this study of the adult human larynx the distribution of mast cells was studied in relation to SP- and CGRP-containing nerve fibers. Substance P– and CGRP-immunoreactive nerve fibers and numerous mast cells were found in the epiglottis and in the subglottic region of the larynx. Only occasional mast cells and no nerve fibers showing SP- or CGRP-like immunoreactivity were found in the vocal cords. In conclusion, the present study has shown that the distribution of nerve fibers showing SP- and CGRP-like immunoreactivity and mast cells has a similar regional variability. As the highest density of SP- and CGRP-containing nerve fibers and mast cells was present in the regions of the larynx where edema occurs, SP, CGRP, and/or mast cells might be involved in the pathogenesis of edema.

KEY WORDS — calcitonin gene–related peptide, edema, human, larynx, mast cells, substance P.

INTRODUCTION

Airway obstruction due to laryngeal edema is often a critical condition. The mechanisms causing edema in the larynx are not fully understood. There is, however, evidence for a functional relationship between mast cells and neuropeptide-containing nerve fibers when both inflammation and edema occur in the airways.[1] In patients with nasal allergy it has been suggested that mast cells located in the nasal mucosa play a role in the onset of the allergic reaction.[2]

Neuropeptides present in sensory nerves are tachykinins such as substance P (SP) and calcitonin gene–related peptide (CGRP). Substance P is known to participate in the neurogenic inflammatory reaction,[3,4] and both SP and CGRP are able to induce histamine release from mast cells.[5,6] In the nasal and bronchial mucosa, allergens and inhaled irritants affect sensory nerve fibers, eliciting a local release of neuropeptides, which in turn is an important factor in the development of the inflammatory reaction.[7]

Recently, we demonstrated the occurrence of a regional distribution of mast cells and SP- and CGRP-containing nerve fibers in the larynx of the rat. The largest number of mast cells and nerve fibers was found in the epiglottic and subglottic regions,[8] ie, the regions in which edema occurs in the larynx of humans. Comparative information on the distribution of peptide-containing nerve fibers and mast cells in the larynx of humans is lacking. Therefore, in the present study the distribution of mast cells in relation to SP- and CGRP-immunoreactive fibers in the human larynx was examined.

MATERIALS AND METHODS

Immunohistochemistry. Small tissue samples from the epiglottis, vocal cords, and subglottic region from seven human larynges were obtained at autopsy. The deceased had no known respiratory impairment. The samples were fixed by immersion overnight in a solution of 4% formaldehyde in 0.1 mol/L phosphate buffer, pH 7.0, at 4°C. They were then rinsed in Tyrode's solution containing 10% sucrose at 4°C for 24 hours, mounted on thin cardboard in water-soluble embedding medium (Miles Laboratories, Naperville, Ill), and frozen in propane chilled with liquid nitrogen.

A series of 10-μm-thick sections were cut, mounted on slides precoated with chrome alum gelatin, dried, and processed for immunofluorescence. Sections stained for nicotinamide adenine dinucleotide–tetrazolium reductase (NADH-TR) activity served as a reference for tissue morphology.[9]

The immunohistologic procedures have been described in detail elsewhere.[10] Briefly, the sections were incubated in Triton X-100, rinsed in phosphate-buffered saline (PBS), pH 7.2, and incubated in

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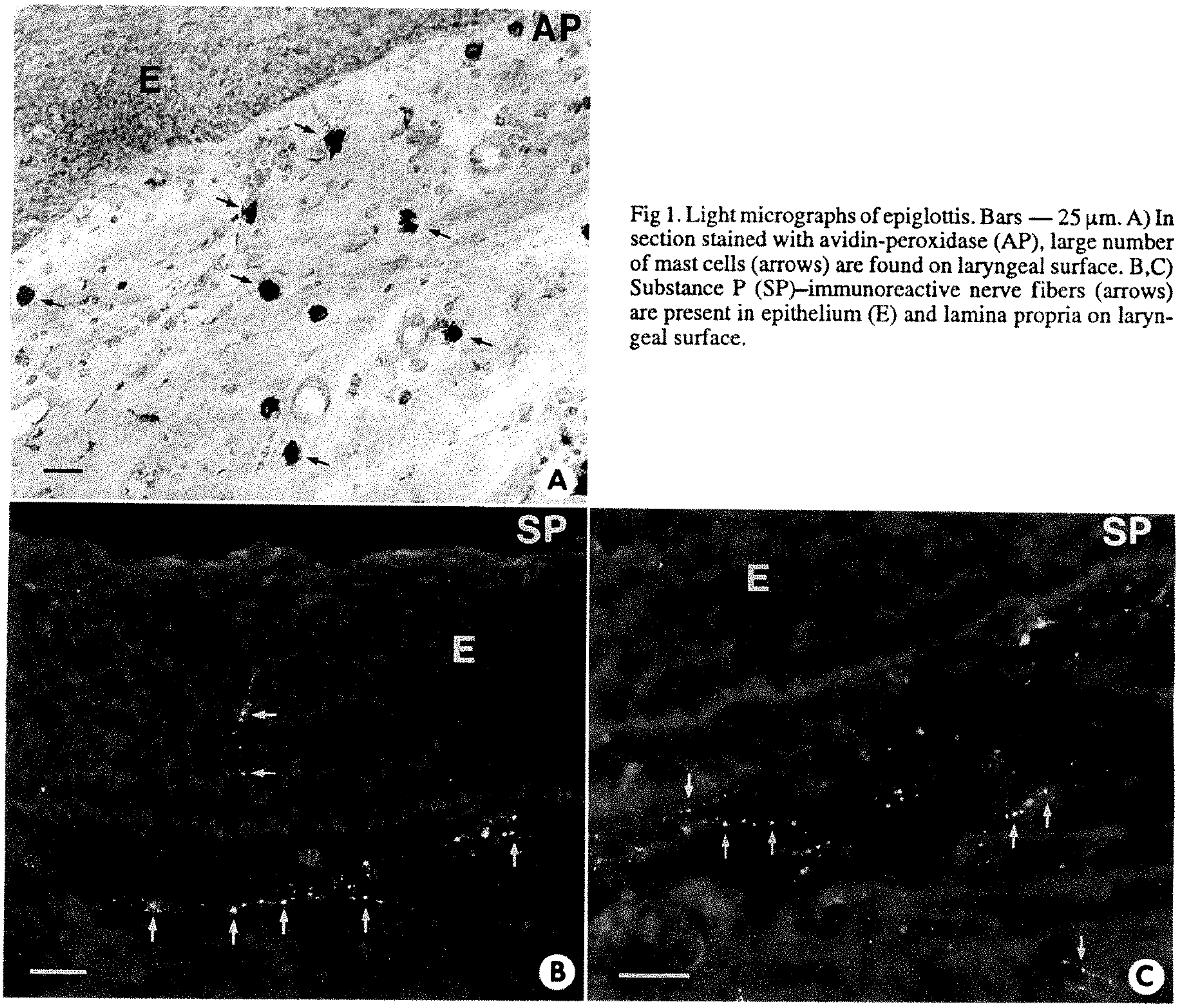

Fig 1. Light micrographs of epiglottis. Bars — 25 µm. A) In section stained with avidin-peroxidase (AP), large number of mast cells (arrows) are found on laryngeal surface. B,C) Substance P (SP)–immunoreactive nerve fibers (arrows) are present in epithelium (E) and lamina propria on laryngeal surface.

normal swine serum. They were then incubated with the primary antibody, washed in PBS, immersed in fluorescein-isothiocyanate (FITC)–conjugated swine anti rabbit IgG, washed in PBS, and mounted in a 1:1 mixture of glycerol and PBS. The sections were examined with a Leitz Orthoplane Photomicroscope equipped with epifluorescence optics. Incubation with the primary antibody was performed for 60 minutes at 37°C, and incubation with the second antibody for 30 minutes at 37°C.

Antisera. Rabbit antibodies against SP and CGRP were used. The SP antibody (code i 675/002, UCB, Brussels, Belgium), raised against synthetic SP conjugated to bovine thyroglobulin, is reported by the supplier not to cross-react with other known neuropeptides, and was used at a dilution of 1:500. The CGRP antibody (code RPN: 1842, Amersham International, London, England), used at a dilution of 1:100, was raised against synthetic α-CGRP conjugated to bovine serum albumin and is reported not to cross-react with other peptides. For control purposes, sections were incubated with SP and CGRP antisera preabsorbed overnight at 4°C with 10 to 20 µg of the respective peptide (Sigma Chemical Co, St Louis, Mo) in 1 mL of antiserum.

Demonstration of Mast Cells. For the demonstration of mast cells, sections from laryngeal tissue fixed as described above were stained with avidin-peroxidase.[11] Parallel sections were processed for SP and CGRP immunoreactivity in order to evaluate a possible topographic association between mast cells and nerve fibers. The fixatives used were Mota's lead acetate,[11] Lison's lead acetate,[12] Carnoy's fixative,[13] and isotonic formaldehyde–acetic acid (IFAA).[14] After fixation, the specimens were dehydrated in a graded series of ethanol and embedded in paraffin. Serial sections (5 µm) were cut and stained with one of the following reagents: 0.1% Alcian blue (pH 5.6), 0.5% toluidine blue (pH 0.5), avidin-peroxidase,[15] or chloroacetate esterase.[16] Mast cells in sections from each anatomic region were counted in three visual fields at 40× magnification, and the number of mast cells per square millimeter was calculated.

RESULTS

Identification of Mast Cells. Mast cells were easy

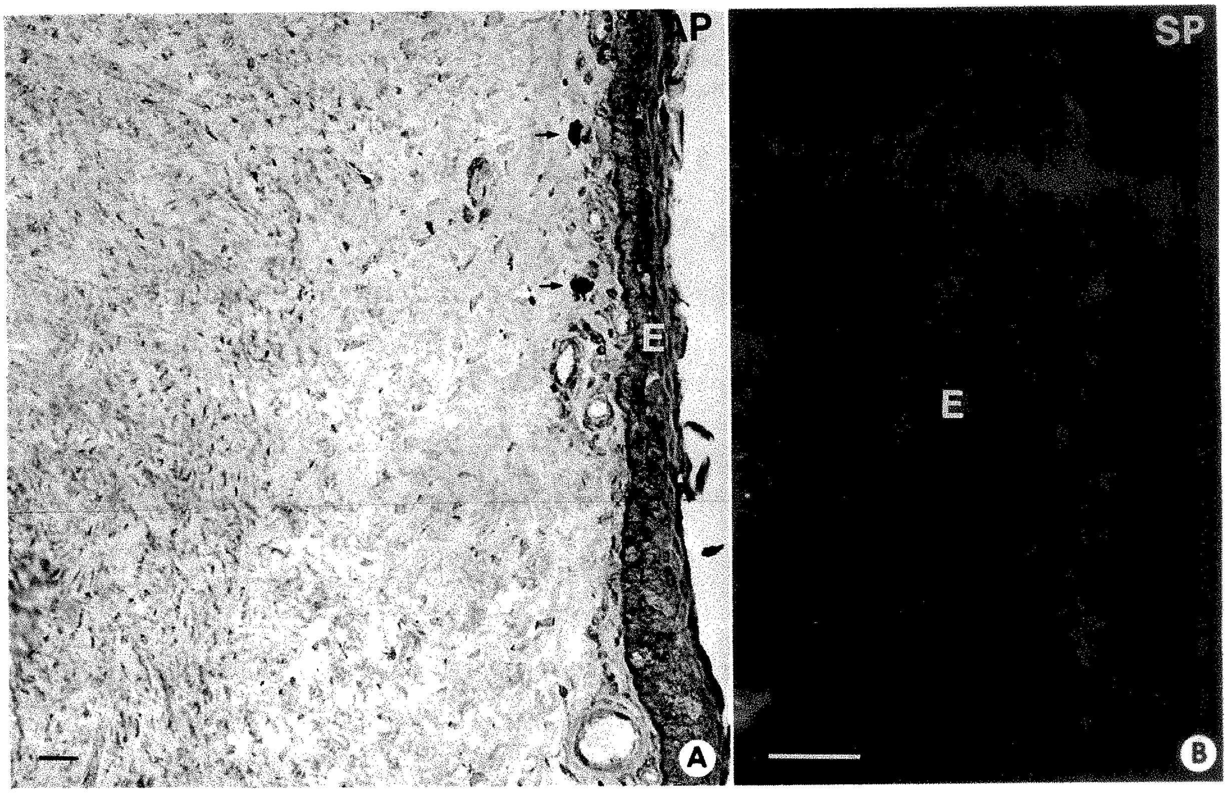

Fig 2. Light micrographs of vocal cord. Bars — 25 μm, E — epithelium. A) In section stained with AP, two mast cells (arrows) are seen in lamina propria. B) Nerve fibers immunoreactive to SP are not found in vocal cord.

to detect in tissues fixed with either formaldehyde or IFAA and stained with avidin-peroxidase (Fig 1A). Although a similar number of mast cells was found with either fixative, tissue morphology was better preserved with formaldehyde than with IFAA. The third-best combination of fixative and staining method was fixation with IFAA, or Lison's fixative, followed by staining with toluidine blue or chloroacetate. The use of Carnoy's fixative resulted in poor morphology, and when Alcian blue stain was used with any of the fixatives, the mast cells were difficult to detect.

Distribution of Mast Cells and Nerve Fibers Showing SP- or CGRP-Like Immunoreactivity. Mast cells were present in large numbers in the lamina propria on both the laryngeal and lingual surfaces of the epiglottis, while the epithelium of the epiglottis was devoid of mast cells (Fig 1A). Nerve fibers showing SP- and CGRP-like immunoreactivity (-LI) were found in the squamous epithelium and particularly in the lamina propria on both surfaces of the epiglottis (Fig 1B,C).

Mast cells were not found in the squamous epithelium of the vocal cords, and only occasionally in the lamina propria of this region (Fig 2A). Nerve fibers displaying SP- or CGRP-LI were not observed in the squamous epithelium or in the underlying lamina propria (Fig 2B).

Mast cells were present in the lamina propria facing the epithelium and deep in the lamina propria but not in the pseudostratified columnar epithelium in the subglottic region (Fig 3A). The number of mast cells in the lamina propria was about half that found in the lamina propria of the epiglottis. Nerve fibers showing SP- and CGRP-LI were observed in both the epithelium and the lamina propria (Fig 3B-D). Furthermore, cells exhibiting SP- or CGRP-LI were detected in the subglottic epithelium (Fig 4).

Nerves, Glands, and Blood Vessels. Large nerve fascicles were present in the lamina propria, and parts of these were composed of fibers displaying SP- or CGRP-LI (Figs 5 and 6A). Mast cells were present in the nerve fascicles (Fig 6B).

A few nerve fibers showing SP- or CGRP-LI were present close to the acini and ducts of the laryngeal glands. No mast cells were found in the glandular tissue (Fig 3A).

The arterioles were supplied with nerve fibers displaying SP- or CGRP-LI. The fibers were often located at the border between the tunica media and the tunica adventitia (Fig 7). Mast cells were frequently found in the vicinity of the arterioles.

Association Between Mast Cells and Nerve Fibers. In the lamina propria of both the epiglottis and the

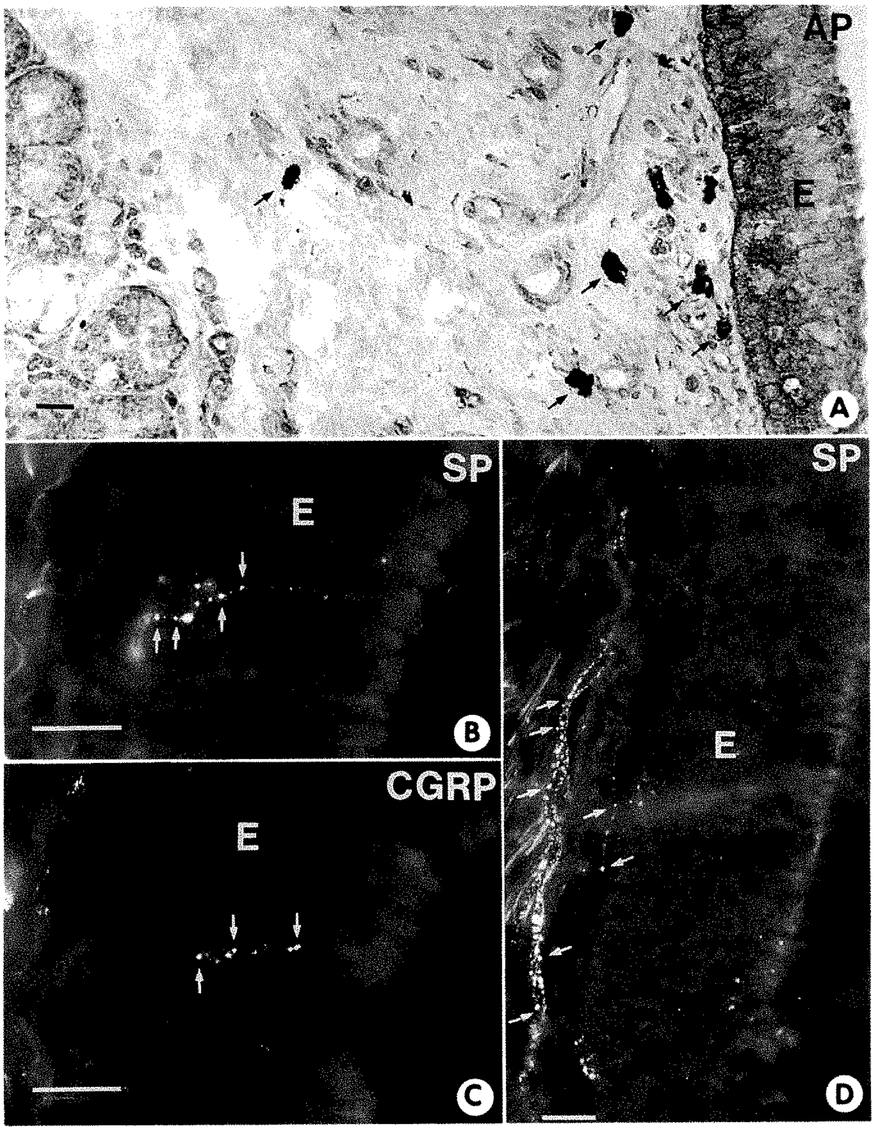

Fig 3. Sections of subglottic region processed for A) AP, B,D) SP immunoreactivity, and C) calcitonin gene–related peptide (CGRP) immunoreactivity. Bars — 25 μm. A) Mast cells (arrows) are found in lamina propria but not in glands. B,D) Nerve fibers immunoreactive to SP are shown in epithelium (E) and lamina propria (arrows). C) Nerve fibers immunoreactive to CGRP (arrows) are seen in epithelium.

subglottic region, mast cells and nerve fibers showing SP- or CGRP-LI were often observed in the same region. A close spatial relationship between the mast cells and the nerve fibers was never observed in these regions, nor was such a relationship noticed in the nerve fascicles (Fig 6) or in the walls of the blood vessels.

DISCUSSION

The present study describes for the first time the distribution of mast cells in relation to nerve fibers showing SP- and CGRP-LI in the larynx of humans. An interesting topographic association between these structures was, moreover, observed. A new observation is also the finding of cells immunoreactive to SP or CGRP in the subglottic pseudostratified epithelium. Cells showing CGRP-LI have frequently been described in the epithelium of the larynx, trachea, and bronchi of several species, including humans.[17-19] These cells have been considered to be neuroendocrine[20] and to have a modulatory and/or chemoreceptive role.[21] Cells showing SP-LI have been found in the epithelium of the peripheral airways in guinea pigs and humans.[22,23]

Mast cells were best visualized when the tissue was fixed with either formaldehyde or IFAA and subsequently stained with avidin-peroxidase. The suitability of avidin-peroxidase as a stain for mast cells may be explained by the fact that mucosal and connective tissue mast cells in humans contain heparinlike glycosaminoglycans[24] and that avidin reacts with heparin.[15] In the rat larynx, heparin-containing connective tissue mast cells, but not chondroitin sulfate–containing mucosal mast cells,[25] can be detected by avidin-peroxidase staining.[8] Chloroacetate esterase staining does not seem to be a method of choice to reveal mast cells in human laryngeal tissue, as fewer cells were detected with this method com-

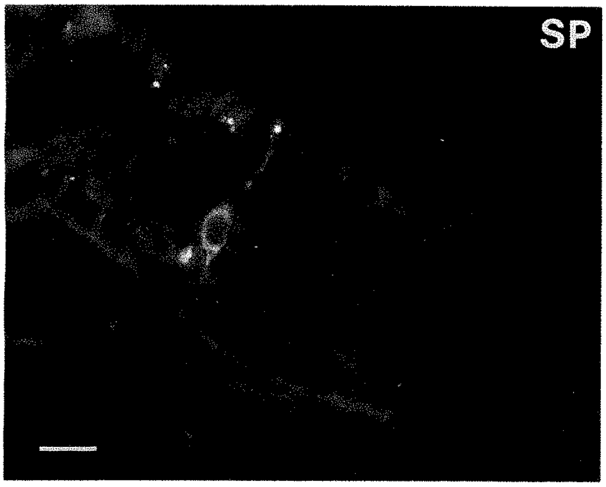

Fig 4. Subglottic region processed for demonstration of SP showing immunoreactive cell within pseudostratified columnar epithelium. Bar — 25 μm.

pared with the avidin-peroxidase staining. Lozewicz et al,[26] who also found fewer mast cells in human bronchi when chloroacetate esterase rather than toluidine blue was used, suggested that the human mast cells are heterogeneous with respect to esterase activity.

There was a large regional variability in the number of mast cells in the lamina propria of the adult human larynx. A large number of mast cells was found in the epiglottis, a moderate number in the subglottic region, and very few were present in the vocal cords. A similar variability in the occurrence of nerve fibers showing SP- and CGRP-LI was found. Thus, in the regions in which mast cells were predominant, ie, in the epiglottic and in the subglottic regions, nerve fibers showing SP- or CGRP-LI were present, while in the vocal cords, where no or few mast cells were present, these fibers were absent. This pattern of covariation resembles that observed in the larynx of the rat.[8] Our observations of SP and CGRP innervation confirm those of Hauser-Kronberger et al,[27] who demonstrated that nerve fibers showing SP- and CGRP-LI occur predominantly in the epiglottic and the subglottic regions in the human larynx.

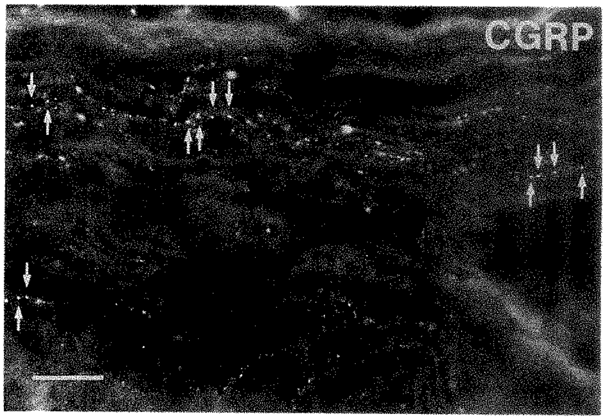

Fig 5. Nerve fascicle processed for demonstration of CGRP showing intraneural immunoreactive nerve fibers (arrows). Bar — 25 μm.

The findings of a high density of SP- and CGRP-containing nerve fibers and mast cells in the lamina propria in the epiglottic and subglottic regions, and the occurrence of such fibers and mast cells in association with blood vessel walls, may have important functional implications. The mast cells, which can be degranulated by SP and CGRP,[5,6] can bind IgE and release mediators known to participate in hypersensitivity reactions[28] and in inflammatory processes. The agents released, or the response elicited, may be modulated by epithelial cells, and the magnitude of the response will depend on the concentrations of the local factors involved.[29] Both CGRP and SP are involved in mechanisms leading to vasodilation and plasma extravasation.[30,31] Substance P may act directly on vessels to produce increased vascular permeability and indirectly, via mast cells and hista-

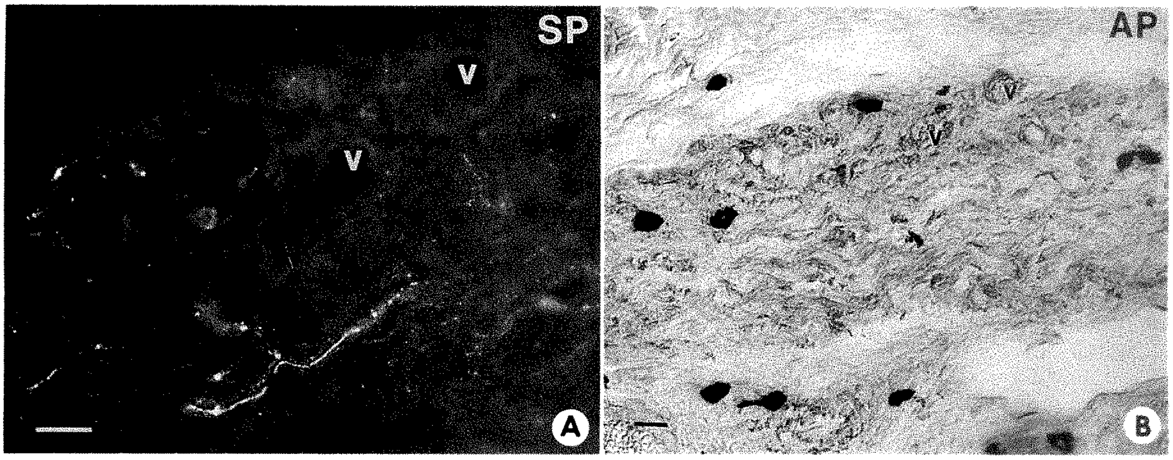

Fig 6. Parallel sections of laryngeal nerve fascicle processed for demonstration of A) SP and B) AP. There is no obvious close proximity between mast cells and immunoreactive nerve fibers. v — blood vessels, bars — 25 μm.

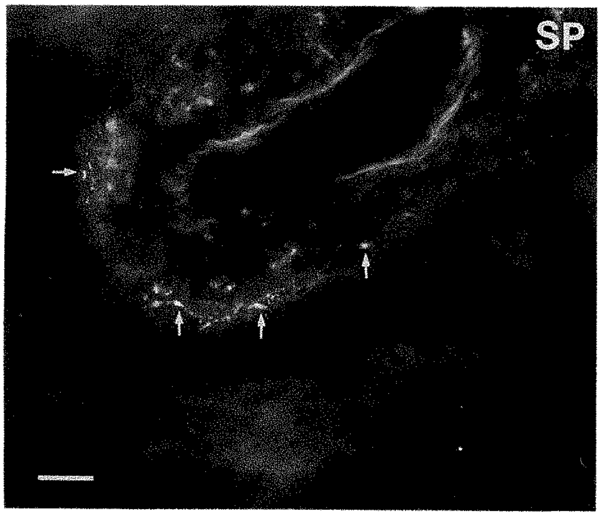

Fig 7. Blood vessel processed for demonstration of SP. Immunoreactive nerve fibers (arrows) are found between media and tunica adventitia. Bar — 25 μm.

mine, to induce vasodilation. A neurogenic inflammatory reaction induced by SP- and CGRP-containing nerve fibers has been shown to occur in the lower airways in response to nerve stimulation and mechanical and chemical irritation.[7,32] In hyperreactive disorders of the respiratory tract, increased vascular permeability is thought to be due partly to activation of sensory nerves and partly to sensory nerve–mediated effects.[33]

The existence of a close topographic association between nerve fibers and mast cells favors the occurrence of neuropeptide–mast cell interactions.[34] In the airways of the pig and rat, mast cells occur in close proximity to fibers showing SP- and CGRP-LI.[8,35] A close spatial association between mast cells and nerve fibers was not, however, observed in the present study. Still, the distances that SP, CGRP, or mast cell mediators are capable of diffusing have not yet been determined; according to some authors,[36] neuropeptides can be transported over long distances.

In conclusion, this study has shown that mast cells are abundant in the lamina propria of the epiglottic and subglottic regions in the adult human larynx and that nerve fibers showing SP- and CGRP-LI are also found in these locations. Thus, there is a morphologic correlate between the occurrence of mast cells and the effects of SP and CGRP in the regions in which edema occurs in the larynx of humans.

ACKNOWLEDGMENTS — Gudrun Furtenbach, Berith Lundström, Ann-Louise Grehn, and Lena Söderberg are acknowledged for skillful technical assistance.

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TWENTIETH ANNUAL MEETING OF THE BÁRÁNY SOCIETY

The Twentieth Annual Meeting of the Bárány Society will be held in Würzburg, Germany, September 20-23, 1998. For more information, contact the Local Organizing Committee, Institute of the 4-G-F, Kurhausstrasse 12, D 97688 Bad Kissingen, Germany; telephone 0049/971-64832; fax 0049/ 971-68637.

Ann Otol Rhinol Laryngol 105:1996

FIBROSARCOMA OF THE THYROID IN A MAN EXPOSED TO FALLOUT FROM THE CHERNOBYL ACCIDENT

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Since the Chernobyl accident in April 1986, a dramatic increase in the incidence of thyroid carcinoma has been described, especially in children. A case of sarcoma of the thyroid in a man exposed to the Chernobyl fallout is presented. Sarcoma of the thyroid is a rare finding. The present patient lived in a region that is known to be one of the most exposed to Chernobyl fallout. The case suggests a high probability of a relationship between the sarcoma of the thyroid and the exposure to radiation.

KEY WORDS — Chernobyl fallout, sarcoma, thyroid.

INTRODUCTION

Since the Chernobyl accident in April 1986, the medical literature has reported a dramatic increase in thyroid cancer, especially in children.[1] Most of the cases are carcinoma, and no sarcoma has been described.[2] We report the case of a man who suffered from thyroid sarcoma 6 years after the accident, who had lived in the Gomel oblast (administrative region) near Chernobyl.

CASE REPORT

A 70-year-old man, recently immigrated to Israel from the Gomel region in Belarus, was admitted in June 1992 to the otolaryngology department of Hadassah University Hospital for a right-sided neck mass that had been developing over 3 months with pain radiating to the right arm, worsening dyspnea, and progressive dysphagia. The patient had undergone partial thyroidectomy 28 years earlier for multinodular goiter and had no history of childhood irradiation. He had suffered from stable angina pectoris for the last decade, treated by nitrates. In the past 2 months there had been a weight loss of 5 kg.

Physical examination revealed a mildly dyspneic, obese man with a pulse of 60/min and a blood pressure of 110/70 mm Hg. There was an old scar (of thyroidectomy) in the anterior neck. In the right side of the neck there was a 10 × 12-cm nontender solid mass with laryngeal deviation to the left. Indirect laryngoscopy revealed a narrowing of the supraglottic area by external compression and a right vocal cord paralysis. On neurologic examination there was a Horner's syndrome and a mild weakness of the right arm with hypoesthesia in the C5-T1 dermatome. No enlarged lymph nodes could be palpated.

Routine laboratory tests were normal except for a urea of 6.7 mmol/L. The level of thyroid-stimulating hormone was 30 mU/L (normal: up to 4 mU/L) with a triiodothyronine level of 1.28 nmol/L (normal: 1.2 to 3.0 μmol/L). Electrocardiography showed a sinus rhythm with no acute or chronic ischemic pattern.

A computed tomography scan of the neck and chest showed an irregular, partially cystic mass arising from the right side of the thyroid gland, with an enhancing irregular rim (Fig 1). The mass extended into the superior mediastinum and the trachea was deviated to the left. Fine needle aspiration was performed but was not conclusive. The patient was taken to the operating room for biopsy. The pathologic examination revealed malignant mesenchymal tumor arising from the thyroid gland, composed of pleomorphic spindle cells, diagnostic of high-grade soft tissue sarcoma. Immunohistochemical stains were positive for vimentin and negative for cytokeratin, desmin, calcitonin, leukocyte common antigen, and chromogranin A. The pathologic conclusion was a fibrosarcoma of the thyroid gland (Fig 2).

Complete surgical excision was judged nonfeasible. Therefore, the treatment was surgical debulking and external irradiation. The patient was discharged 1 week after the debulking operation, to be followed up by the radiotherapy oncologist. A few days after the beginning of the radiotherapy the patient developed an acute anterior wall myocardial infarct, and he died several days afterward.

From the Departments of Otolaryngology–Head and Neck Surgery (Sichel, Dano, Elidan), Oncology (Wygoda), and Pathology (Osin), Hadassah University Hospital, Jerusalem, Israel.

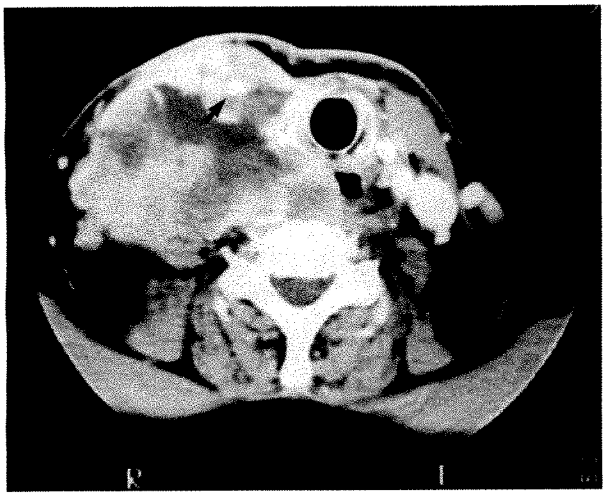

Fig 1. Computed tomogram in axial plane showing huge tumor developing from thyroid gland, reaching spine and engulfing common carotid artery (arrow).

DISCUSSION

The data on radiation-induced thyroid cancers in humans come from several sources, including Japanese atomic bomb survivors,[3] Marshallese exposed to radioactive fallout,[4] children irradiated for benign conditions,[5,6] and long-term survivors of therapeutic irradiation.[7,8]

In most of these studies, a latency period of 10 years or more was observed between the ionizing radiation exposure and the diagnosis of thyroid cancer. This is why the increasing incidence of thyroid cancers occurring 4 years after the Chernobyl accident raised the question in the medical community of the thyroid cancers' link with the explosion. Meanwhile, this link has statistically been clearly established: from 1978 to 1985 there was a total of 5 cases of thyroid carcinoma in children reported in Belarus; from 1986 to 1993, 251 new cases were reported, 135 of them in the Gomel region.[2] In adults, also, there has been an increased incidence of thyroid cancer reported in Belarus since the Chernobyl accident: 554 cases between 1982 and 1985; 797 between 1986 and 1989; and 1,557 between 1990 and 1993.[2] To the best of our knowledge none of those cancers were sarcoma. In 244 of the 251 surgically treated children the pathology was papillary carcinoma (229 or 93.9%), follicular carcinoma (14 or 5.7%), or medullary carcinoma (1 or 0.4%).[2]

However, the relationship between ionizing radiation and sarcomas is well known and the development of thyroid radioinduced sarcoma has been occasionally described.

Griem et al[9] in 1989 published a case of a 23-year-old man who developed a sarcoma of the thyroid 12 years after neck irradiation for lymphoepithelioma at the nasopharynx. The thyroid gland was irradiated in this patient to a total dose of 7,000 cGy.

Of interest, Marks et al[10] did an experiment exposing sheep thyroid glands to ionizing radiation by means of daily feedings of small quantities of iodine 131 for the duration of 5 years. The only definitely malignant tumor encountered was a fibrosarcoma of the thyroid observed at the age of 53 months in a ewe exposed to a level of 5 mCi daily since its fetal life.

Our patient lived in Gomel at the time of the Chernobyl accident. Precise measurements of the activity of the iodines were not taken after the explosion due to their short periods, but a cartography of cesium 137 is available and it is thought that this should be very similar to the cartography of the iodines. In view of this, it is estimated that the

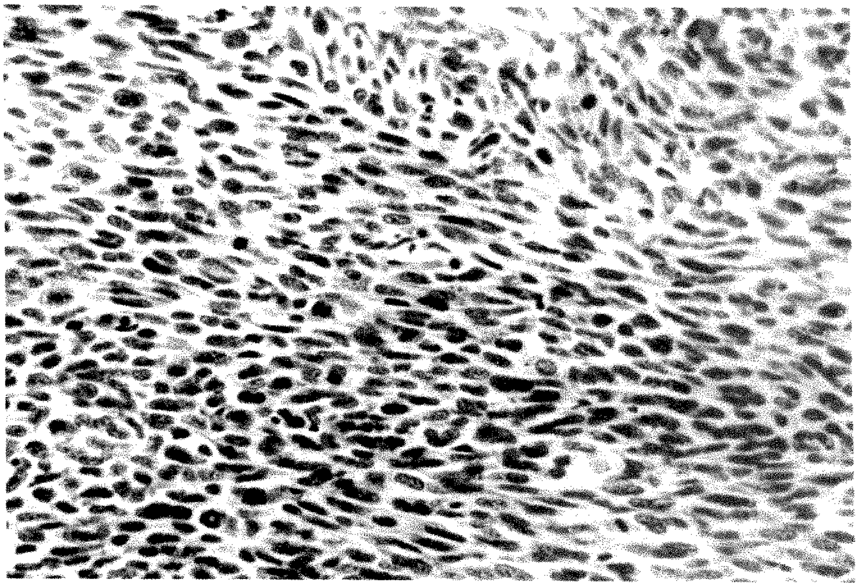

Fig 2. Fibrosarcoma, composed of spindle cells, displaying high degree of cellularity, prominent nuclear anaplasia, and numerous mitotic figures (H & E, original ×250).

contamination by iodine 131 in the Gomel region was higher than 150 Ci/km^2. (The highest level of contamination of iodine 131 registered in Belarus was 1,500 Ci/km^2.) Among the six oblasts of Belarus, Gomel is by far the one in which the increase in the incidence of thyroid carcinoma has been the most drastic.[11]

In conclusion, we presented a case of sarcoma of the thyroid arising 6 years after the Chernobyl accident in an adult who lived in the Gomel oblast at the time of explosion. In view of the extreme rarity of thyroid sarcomas and of the very high dose of radioactive fallout in this region, we believe that this case is to be considered radioinduced.

As already noticed by Baverstock et al,[12] the carcinogenic effect on the human thyroid of radioactive fallout is much greater than previously thought, and the future will tell us whether, as for differentiated carcinomas of the thyroid, an increase in the incidence of thyroid sarcomas will be observed.

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EVALUATION AND MANAGEMENT OF THE PATIENT WITH DIZZINESS

The course Evaluation and Management of the Patient With Dizziness will be held in New York, New York, November 1-2, 1996. For more information, contact New York University, 550 First Ave, New York, NY 10016; telephone (212) 263-5295; fax (212) 263-5293.

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IMAGING CASE STUDY OF THE MONTH
PLEOMORPHIC ADENOMA OF THE TONGUE BASE

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INTRODUCTION

Involvement of the base of the tongue by pleomorphic adenoma is an uncommon and not frequent condition. It represents a therapeutic challenge. This report presents our experience in managing a patient who was found to have a large pleomorphic adenoma of this anatomic structure and discusses the subsequent diagnosis and treatment.

CASE REPORT

An 82-year-old woman was referred to our department complaining of moderate dysphagia. The symptom developed over a period of 1 year with progressive exacerbation. She had no pain on swallowing.

Examination of her oral cavity showed a lesion in the base of the tongue. Fiberoptic direct laryngoscopy revealed a mass extending toward the epiglottis without displacing it. The tumor measured approximately 3 x 4 cm, was located on the right side, and partially overcame the midline. The valleculae were not affected. The surface was irregular, with a grayish tumoral appearance. Normal movement of the tongue was detected. Magnetic resonance imaging (MRI) of the head and neck was performed and disclosed a tongue mass with minimal involvement of the underlying muscles. The tumor had a solid component (Figs 1 and 2). No enlargement of the lymph nodes was observed in the neck. Local biopsy of the tumor was advised. Histologic examination revealed a benign pleomorphic adenoma (Fig 3).

Because of the age of the patient and the benign nature of the lesion, we planned a partial removal of the mass in order to improve the symptoms.

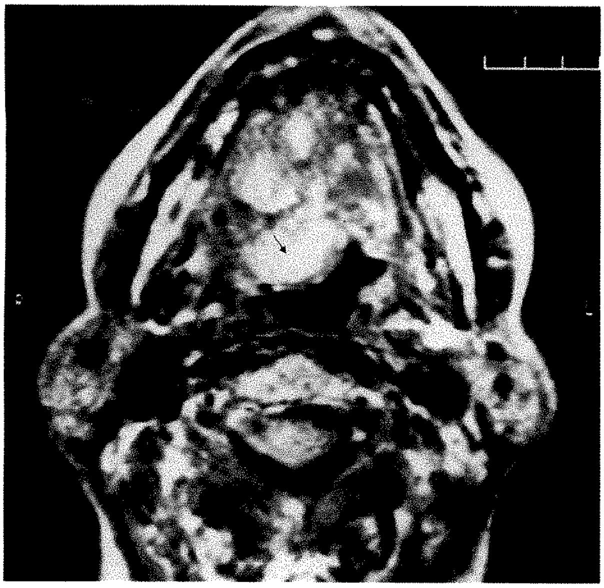

Fig 1. Axial magnetic resonance image reveals lesion (arrow) in base of tongue. Shows minimal involvement of underlying muscles.

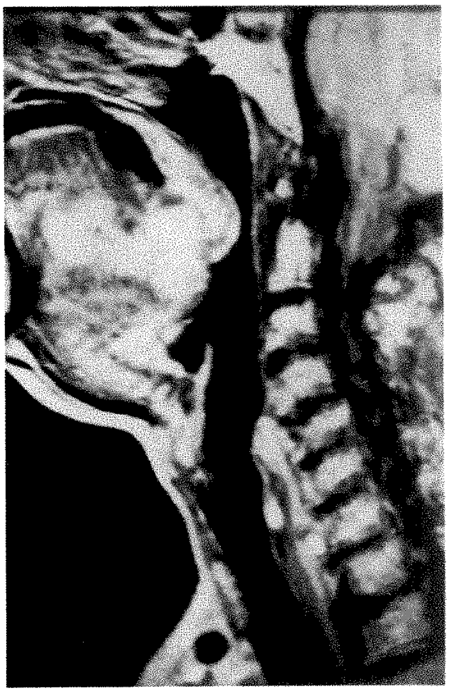

Fig 2. Sagittal coronal view reveals solid lesion extending toward epiglottis without displacing it.

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REPRINTS — Giuseppe Magliulo, MD, Via Gregorio VII, 80, 00165 Roma, Italy.

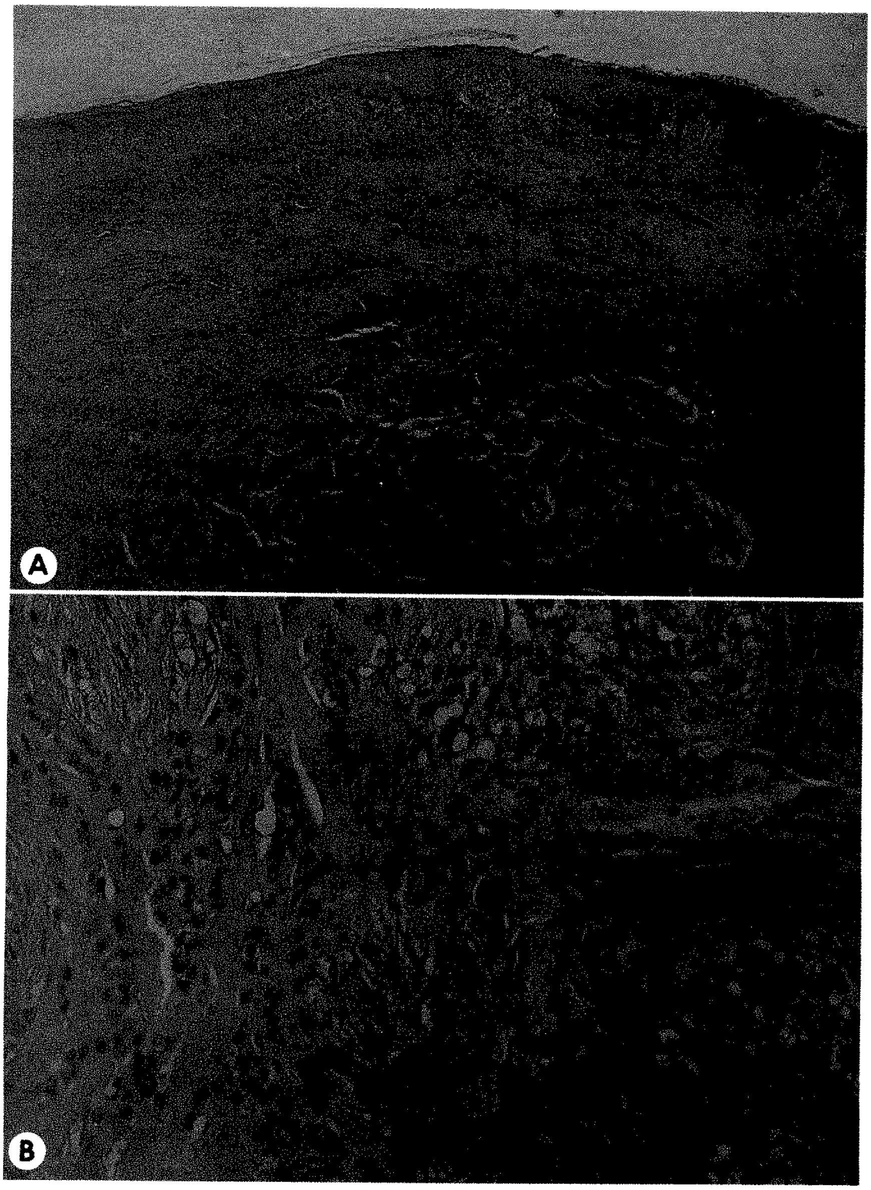

Fig 3. Benign pleomorphic adenoma. A) Lesion is not well circumscribed and is localized under epithelia (original ×4). B) Lesion shows clear appearance of both stromal and myxoid components (original ×25).

The patient underwent a carbon dioxide laser tumor removal. Although the tumor was partially eradicated, the wound healed rapidly by second intention. The adenoma persisted after 3 years of follow-up, but the patient was symptom-free. On MRI the tumor was seen to remain stable with no enlargement.

DISCUSSION

The occurrence of a benign pleomorphic adenoma of the base of the tongue is rare. A review of the available literature revealed that fewer than five cases have been reported to date.[1-6] Their incidence among the minor salivary gland tumors is variable between 2% and 2.4%.[1,3] It should be pointed out that minor salivary gland tumors represent only 9% of all salivary tumors.[1] Diagnosis of this rare pathologic condition is relatively easy, being assured by inspection of the oral cavity and direct laryngoscopy. Its histologic confirmation is obviously given by local biopsy.[7]

Imaging studies, particularly MRI for its higher resolution power of the soft tissues, provide useful information on the nature of the lesion (benign or malignant) with specific regard to the involvement of the lymph nodes. They are also excellent in delineating the extent of the tumor and the status of the surrounding structures.

The treatment of choice of a pleomorphic adenoma of the base of the tongue is surgical therapy, but this depends on various parameters. First, the site and size of the tumor might require more extensive surgery for its total eradication. Different surgical approaches are recommended (lateral pharyngotomy, midline transhyoid approach, median translingual pharyngotomy, anterior mandibulotomy).[8]

The old age and bad general condition of our patient obliged us to tailor a less aggressive surgical strategy, preferring a partial enucleation of the tumor. Such management did not adversely influence the clinical evolution of our patient, who remained symptom-free at 3 years' follow-up.

A favorable outcome was confirmed by the absence of tumor regrowth. Should the tumor provoke worsening of symptoms, another rationale for treatment (radical surgical excision or irradiation) linked to the specific situation of each case would have to be considered.

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SUPRAESOPHAGEAL COMPLICATIONS OF REFLUX DISEASE

Supraesophageal Complications of Reflux Disease will be held November 21-23, 1996, in Tucson, Arizona. For further information, contact Reza Shaker, MD, MCW Dysphagia Institute, Division of Gastroenterology, Froedtert Memorial Lutheran Hospital, 9200 W Wisconsin Ave, Milwaukee, WI 53226; telephone (414) 259-2742; fax (414) 384-8480.

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PATHOLOGY CONSULTATION

EWING'S SARCOMA AND PERIPHERAL PRIMITIVE NEUROECTODERMAL TUMOR: AN INTERIM REPORT

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The primitive neuroectoderm and its progeny seemingly give rise to an ever-increasing number of clinicopathologic entities. Ewing's sarcoma and peripheral primitive neuroectodermal tumor have recently been united by relatively unique antigens expressed by the MIC-2 gene, commonly coexpressed neural markers, and cytogenetic and molecular genetic abnormalities. Because of these factors, the current thinking is that Ewing's sarcoma and the peripheral primitive neuroectodermal tumor are parts of a phenotypic spectrum. We present an "interim report" on this group of neoplasms, emphasizing their presentation in the head and neck.

The concept of a primitive neuroectodermal tumor (PNET) has been the subject of considerable debate and an abundant literature.[1-4] At first, the term was limited to tumors of the central nervous system (CNS), where they were traditionally called medulloblastomas and preferentially located in the cerebellum.[5] The concept was expanded to peripheral, histologically similar, non-CNS tumors and they were called peripheral PNETs (pPNETs), as opposed to central PNETs (cPNETs).[5] With time, each group underwent subdivisions, the most remarkable being in the pPNETs (Fig 1). The classic peripheral neuroblastoma is much more closely related to the cPNETs than the pPNETs, in that it and cPNETs partially recapitulate the molecular phenotype of immature neurons and other precursor cells at different stages in their development. Significant differences exist, however, in their chromosomal abnormalities and gene amplification. Peripheral neuroblastomas are characterized by deletions of the short arm of chromosome 1(1p-) and by frequent amplifications of N-myc gene.[5] Central PNETs show frequent changes of chromosome 17 and only a limited gene amplification.[5]

There is a close relationship between Ewing's sarcoma (ES) and pPNETs, including Askin's tumor, but only a distant relationship to neuroblastoma.[2,3] The ES-pPNET group always lacks N-myc expression, unlike nearly half of neuroblastomas and all true neuroblastomas that grow in vitro.[2,6] Also, unlike neuroblastomas, ES-pPNETs are not considered to be catecholamine producers.[2,6] The cytogenetic translocations seen in ES-pPNETs are not found in neuroblastomas (see below). There is an absence of HLA class I antigen expression, eg, β2-microglobulin, by neuroblastomas, but an abundant expression by ES.[3] Cell lines from ES, under normal culture conditions, do not give evidence of a neural differentiation.[3] Treatment with retinoic acid, known to induce neural differentiation in cultured neuroblastomas, fails to do so with ES cells. On the other hand, there is a pronounced neural differentiation by ES cell lines treated with cyclic adenosine monophosphate (AMP) or phorbol 12-myristate 13-acetate (TPA).[3]

A relatively unique antigen expressed by ES is the MIC-2 gene and its glycoprotein product p30-32.[7] Antibodies raised against the protein can demonstrate it in over 90% of the ES-pPNET group.[8] The product of the MIC-2 gene is involved in cellular

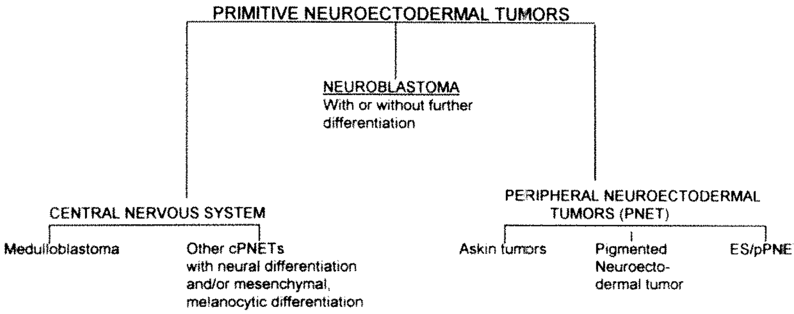
PRIMITIVE NEUROECTODERMAL TUMORS
NEUROBLASTOMA
With or without further differentiation
CENTRAL NERVOUS SYSTEM
PERIPHERAL NEUROECTODERMAL TUMORS (PNET)
Medulloblastoma
Other cPNETs with neural differentiation and/or mesenchymal, melanocytic differentiation
Askin tumors
Pigmented Neuroectodermal tumor
ES/pPNET

Fig 1. Subdivision of primitive neuroectodermal tumors into three major groups. Peripheral group is further divided into at least three subtypes. ES — Ewing's sarcoma.

From the Department of Pathology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas.

TABLE 1. IMMUNOCYTOCHEMICAL DIFFERENTIAL DIAGNOSIS OF SELECTED "SMALL, BLUE, ROUND CELL TUMORS" OF HEAD AND NECK

	Percent of Tumors With Antigens				
Tumor	*p30-32 MIC-2*	*NF*	*Myogenin*	*Desmin*	*MSA*
ES-pPNET	96	23	0	12	0
Neuroblastoma	0	83	0	0	0
Rhabdomyosarcoma	3	0	95	100	91

Modified from Wang et al.[9]

NF — neurofilaments, MSA — muscle-specific actin, ES — Ewing's sarcoma, pPNET — peripheral primitive neuroectodermal tumor.

adhesion processes, but the gene is not associated with the translocations considered specific for this group of tumors. It is an independent characteristic, but is not a tumor-specific antigen, since it is normally expressed as a component of the T-cell receptor complex. As may be seen in Table 1,[9] the demonstration of MIC-2 gene products is a rather strong indicator of the ES-pPNET group of neoplasms.[9] This view is shared by Grier,[10] Lee et al,[11] Jürgens,[12] and Kovar et al,[13] despite its presence on lymphoid, ependymal, and endocrine cells.[14]

EWING'S SARCOMA AND PRIMITIVE NEUROECTODERMAL TUMORS IN HEAD AND NECK

In the head and neck, after exclusion of a nodal or extranodal lymphoma, the differential diagnosis of small, blue, round cell tumors centers on rhabdomyosarcoma, primary or secondary neuroblastomas, and the ES-pPNET tumor group. Because of the too-frequent limitations of hematoxylin and eosin light optic examination, immunohistochemical and electron optic studies are necessary for resolution. Neither technique is capable of 100% accuracy, specificity, or sensitivity.

Differential Diagnosis. Since our concern in this report is with the ES-pPNET group of neoplasms, we will only briefly address the elimination of rhabdomyosarcomas from the differential diagnosis. The recent recognition of the role of myogenic regulatory proteins in skeletal muscle development and differentiation has provided monoclonal antibodies to augment the current standbys — desmin and muscle-specific actins.[15] The myogenic regulating proteins of the myogenic determination family play a critical role in the commitment of mesenchymal progenitor cells to a myogenic lineage and then the differentiation and maintenance of a skeletal muscle phenotype.[9,15] The regulating factors also enjoy a temporal expression advantage in that they (myogenin and others) are expressed much earlier in the differentiation of normal skeletal muscle than desmin, actin, or myosin.[15]

TABLE 2. CYTOGENETIC TRANSLOCATIONS AND ASSOCIATED FUSION GENES IN THREE SOFT TISSUE SARCOMAS

Neoplasm	*Translocations*	*Fusion Genes*
ES-pPNET	t(11;22)(q24;q11.2-12)	EWS/FLI-1
	t(21;22)(q22;q12)	EWS/ERG
Clear cell sarcoma	t(12;22)(q13-14;q12)	EWS/ATF-1
Desmoplastic small round cell tumor	t(11;22)(p13;q12)	EWS/WT-1

Modified from Bridge.[16]

Wang et al,[9] using a heat-induced epitope unmasking technique and deparaffinized, formalin-fixed tissues, compared the sensitivity and specificity of several antibodies, including those against myogenin and muscle-specific actin, in an immunohistochemical study of rhabdomyosarcoma, ES and pPNET, and neuroblastoma (Table 1). Although the sensitivity of antimyogenin is slightly less than that of antidesmin in the Wang et al[9] study, the specificity of myogenin is better than that of antibodies to desmin; eg, desmin antibodies do not distinguish between skeletal and smooth muscle differentiation, and desmin expression has been noted in nonmyogenous neoplasms.[9]

Cytomolecular Genetics. That ES and pPNET are histogenetically related tumors has been amply demonstrated, not only by ultrastructural findings and immunohistochemical similarities, but also by a sharing of unique chromosomal translocations.[16] Until more distinctions between the two neoplasms can be defined, it is probably best to consider ES and pPNET

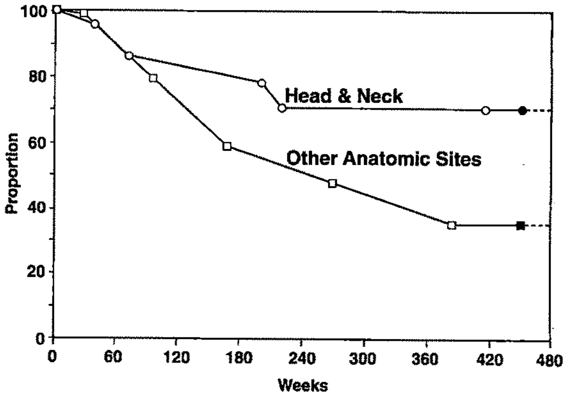

Fig 2. Survival of patients with Ewing's carcinoma of gnathic bone (head and neck) compared to all other osseous sites. Modified from Siegal et al.[28]

TABLE 3. EXTRAOSSEOUS EWING'S SARCOMA

Sex: Slight male predominance
Age: Mean age at diagnosis = 11.2 years
Primary site (%)

Trunk	32
Extremities	26
Head and neck	6
Parameningeal	9
Orbit	2
Intrathoracic	3
Pelvis	2
Genitourinary	1
Other	1

Adapted from Shimada et al.[29]

TABLE 4. SERIES TABULATION OF PERIPHERAL PRIMITIVE NEUROECTODERMAL TUMORS

Anatomic Location	*Cases* *No.*	*%*
Thoracopulmonary*	12	46
Head and neck†	11	42
Other	3	12

N = 26; data extracted from Jones and McGill.[24]

*Paravertebral 8, chest wall 4.

†Neck 3, parotid gland 1, larynx 1, scalp 1, mandible 1, pterygomaxillary space 1, paranasal sinus 1, nasal cavity 1, retropharynx 1.

as the opposite ends of a spectrum of a closely related group of tumors called ES-pPNET.[1,4,17]

The most compelling evidence linking ES and pPNET is the cytogenetic translocation t(11;22)(q24; q11.2-12), in 85% to 90% of the tumors analyzed.[16] As a consequence of this translocation, the 5' portion of the EWS gene from band 22q12 is fused to the 3' portion of the FLI-1 gene from band 11q24.[18,19] The fusion results in the formation of a hybrid EWS–FLI-1 protein. This EWS–FLI-1 contributes to the transformed phenotype of ES cells by inducing a deregulated activation of genes, or an unscheduled activation of genes, or both.[18,19] In addition to the EWS–FLI-1 fusion, the EWS gene can also be fused to ERG, another transcription factor closely related to FLI-1, but found on chromosome 21 and yielding another cytogenetic variant — t(21;22)(q22;q12).[19] Both FLI-1 and ERG gene products are regulators of transcription activators.

According to Bridge,[16] no fully typical ES or pPNET lacks an EWS–FLI-1 or an EWS-ERG fusion transcript.

The promiscuous nature of the EWS gene on chromosome 22 is amply demonstrated by its participation in four separate translocations (Table 2[16]) associated with three separate tumors: the two members of the ES-pPNET group, desmoplastic small round cell tumor, and clear cell sarcoma (also called amelanotic melanoma of soft parts).[16] The ESs and pPNETs also share a consistent pattern of proto-oncogene expression, ie, c-myc, n-myc, c-myb, c-mil–raf1, and db1.[4,16]

In summary, the ES-pPNET group is characterized by reciprocal translocations that result in the formation of chimeric genes having the properties of transcriptional regulators. The outcome is an expression of similar disease (neoplastic) phenotypes.[16]

Histogenesis. The ES-pPNET group comprises osseous and nonosseous ES, malignant peripheral neuroectodermal tumor of bone and soft tissues, the clinical and topographic variant of malignant peripheral neuroectodermal tumor occurring in the thoracopulmonary region, known as Askin's tumor, pigmented neuroectodermal tumor, ectomesenchymoma, and the very rare peripheral medulloepithelioma. We have excluded olfactory neuroblastoma because of contradictory findings.[20]

Current thinking places the osseous ES as the most common and least differentiated pPNET, with the others falling in a spectrum of the same basic neuroectodermal tumor system with various degrees of neural differentiation.[3,21,22] This thesis presupposes a common histogenesis that, unfortunately, is difficult to prove. In that regard, the rounding up of the usual suspects, the neural crest cells, does not accom-

TABLE 5. IMMUNOHISTOCHEMICAL PROFILE OF ES-pPNET

Antibody	*ES*	*pPNET*
Cytokeratin	Positive on occasion (less than 5%)	Positive on occasion (up to 10%)
Neurofilaments	Negative	Positive on occasion
Glial fibrillary acidic protein	Negative	Positive on occasion
Neuron-specific protein	Negative	Positive
S-100 protein	Negative	Positive in about 50% of cases
Leu 7	Negative	Positive in about 30% of cases
Chromogranin	Negative	Positive on occasion
Antibodies against MIC-2 gene product	Positive	Positive
Desmin	Negative	Positive on occasion

Modified from Schmidt.[31]

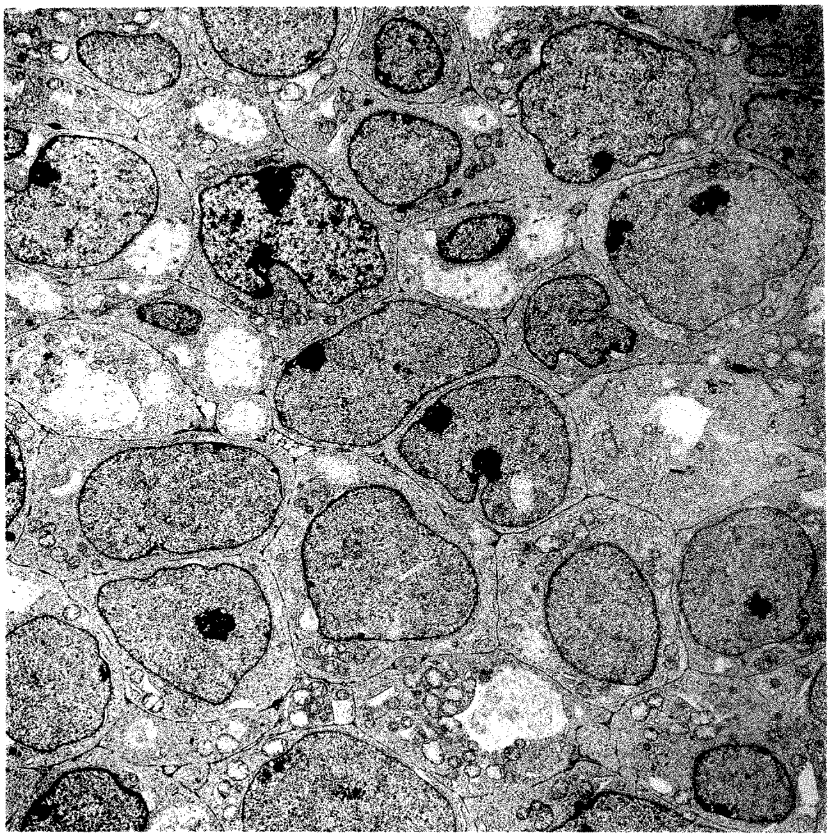

Fig 3. Cells of Ewing's tumor of bone have smooth surfaces that tend to be intimately apposed, but cell junctions are scattered, tiny, and primitive, and they can barely be identified at low magnification (original ×4,200). In this classic example, nuclei have smooth contours and small nucleoli. Some mitochondria can be seen, but main feature of cytoplasm is electron-lucent zones that are lakes of glycogen.

modate all of the tumors; nor does neuroectoderm, the forebear of crestogenic cells. Only in the cephalic region do the neural crest cells have an ectomesenchymal potential, and the head and neck is not an anatomic region of predilection for either ES or pPNETs.[23,24] Neuroblastomas are considered neoplastic derivatives of the neural crest, but they are only remotely related to the ES-pPNET group. Unexplained, also, is the usually benign clinical course of the pigmented neuroectodermal tumor of the anterior maxilla, if it is a member of a group of tumors typically characterized by a highly malignant course. The removal of the neural crest theory leaves us with the equally non–intellectually satisfying "uncommitted pluripotential cell" that can express a neural phenotype, as well as epithelial and myogenic differentiation.[25]

Ewing's Sarcoma of Jaws. Ewing's sarcoma accounts for 4% to 7% of all primary bone tumors.[26] The long bones of the extremities and the pelvis comprise 58% and 20% of all documented cases, respectively.[27] The ribs rank third in frequency. In contrast, only 3% arise in the skull and jaw bones.[27] Metastasis of an ES to jaw bones is rare, representing 0.2% to 1.5% of all reported cases of the tumor.[27] The mandible, particularly the posterior part, has a 2:1 higher incidence of primary origin than the maxilla.[26]

In 1990, Wood et al[26] recorded the 105th case of ES involving jaw bones. Primary lesions exceeded metastatic lesions 98 to 7 (14:1). The average age at diagnosis was 15.9 years with a range of 2 to 44 years. There was nearly an equal involvement of sexes. Ewing's sarcoma of the jaws is predominantly a neoplasm of white persons and is rare in blacks. Swelling of the afflicted area, often accompanied by pain, is the most frequent complaint. A laminar periosteal response by itself is not a feature of this disease in the jaw bones. A common radiologic feature is that of a poorly defined osteolytic lesion, which may have some sun-ray spicules of periosteal bone. Other common signs include a soft tissue mass adjacent to the destructive site and a destruction of cortices of follicles or unerupted teeth.

Location of ES of bone is considered the single most important predictor of clinical behavior; eg, long bone primaries are associated with a significantly better prognosis than those in the pelvis.[28] Gnathic bone ES is credited with a significantly lower mortality compared with all other primary sites (Fig 2[28]). A primary involving cervical vertebrae, however, is associated with a very poor outlook.[28]

Before multiagent chemotherapy, the overall 5-year survival for ES of bone was 16%, and of the survivors, one fourth died of their disease after 5 years. Integrated therapies, including surgery, irradiation, and multiagent chemotherapy, have improved

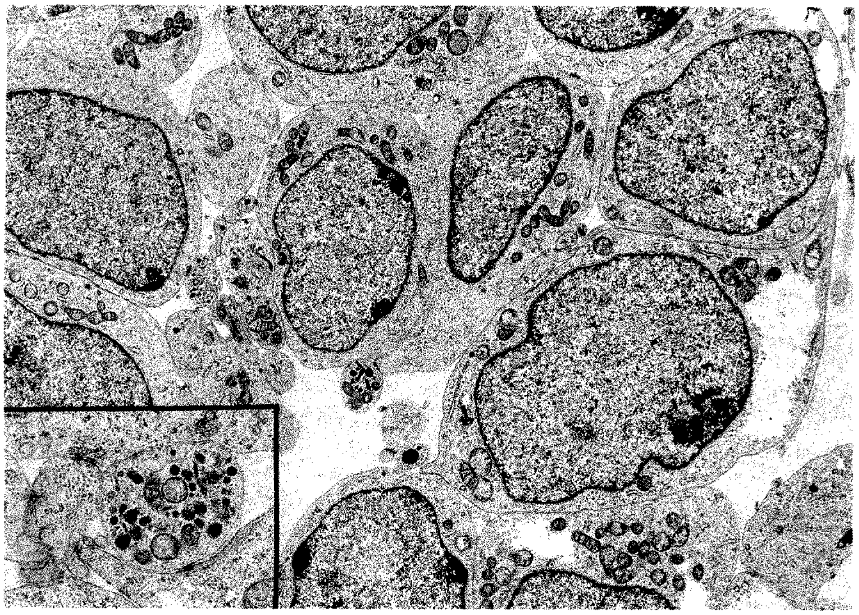

Fig 4. Cells of this peripheral neuroectodermal tumor resemble those of Ewing's tumor, but short dendritic processes lie between cells, individually or in small groups (original ×7,400). Inset) Two adjacent processes in cross section (original ×14,000). Larger one contains some mitochondria and dense-core granules; small circles in other process are microtubules.

the 5-year survival for localized disease to 54% to 74%.[28]

Extraosseous Ewing's Sarcoma–pPNET of Head and Neck. Extraosseous ESs, some but not all of which have an association with a large nerve, are not without controversy over histologic criteria. Nonetheless, several series, dealing with a uniform collection of tumors, have provided accepted clinicopathologic assessments. Shimada et al,[29] after a pathology review of the Intergroup Rhabdomyosarcoma Study I and II cases, reported on 84 cases of extraosseous ES. Table 3[29] lists several clinical aspects of these cases. The primary site designations are those of the Intergroup. Combining Intergroup regions, "head and neck, parameningeal and orbit," the number and percent of extraosseous ES cases in the head and neck are 15 and 17.8%, respectively. This is considerably higher than the 4.8% reported by Rud et al[30] in their analysis of Mayo Clinic patients. Rud et al also found extraosseous ES to be an aggressive disease with high rates of local recurrence and distant metastases. The overall 5-year survival of 48% in their patients treated after 1970 speaks to the lethality of the tumor. The average survival after pulmonary metastases in the Mayo Clinic patients was 10 months. Rud et al conclude that neither surgery alone nor chemotherapy and irradiation alone is sufficient for achieving a long-term disease-free survival. In every case, adjuvant multiagent chemotherapy should be considered, since ES is viewed as a systemic disease even when it is clinically localized.

Because of the relatively recent delineation of criteria for the diagnosis of pPNET, the incidence is not possible to state. It is possible that as many as half of tumors previously diagnosed as ES are pPNETs.[24]

Peripheral PNETs can occur in any age group, with a peak in adolescence (median age 11 to 22 years).[24] There is no sex predominance. In a report from Children's Hospital and the Dana Farber Cancer Institute, Boston, Mass, 11 of 26 pPNETs were in the head and neck (Table 4[24]). The tumors manifest a rapid clinical progression. Metastases are often noted at the time of diagnosis (approximately 30%).[24]

A multimodality regimen, like that for ES, is the current therapy. Even with this, survival is poor. Jones and McGill[24] report that only 65% of their patients were alive within 2 years of diagnosis. Metastasis at diagnosis further decreases survival.

CONCLUSIONS

We have indicated in the title of this report that it is an interim report. This is an acknowledgment that the concept of a PNET is still evolving. From a molecular biology standpoint, the recent findings are exciting. From a practical diagnostic and therapeutic perspective, however, is the separation of the ES-pPNET group into separate clinicopathologic entities of any advantage?

Table 5[31] presents an immunohistochemical profile of ES and pPNETs. The use of the term "on occasion" is unqualified as to frequency and refers only to immunophenotypes possibly found in any given tumor. There is immunoreactivity overlap.

Electron optic identification of neurosecretory-type granules associated with microtubules and intermediate filaments, presumably neurofilaments, separates the "classic" ES from a pPNET[31] (Figs 3 and 4).

The prognostic significance of neural differentiation seen in pPNETs is unsettled, despite allusions that survival rates in pPNETs are lower than in ES. The comparability of therapeutic strategies is another indicator that any current separation of the ES-pPNET group does not provide an advantage.

There is a possibility that additional cytogenetics can detect patients with very aggressive tumors. Sainati et al,[32] for example, have found that deletion or rearrangement of all or part of the short arm of chromosome 1 in pPNETs is associated with a poor outlook and general nonresponsiveness to chemotherapy.

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Color Atlas of Oral Diseases

Second edition. George Laskaris. Hard cover, indexed, illustrated, 386 pages, 1994. Thieme Medical Publishers, New York, NY, $99.

This second edition expands the previous edition published in 1988 by including new chapters and illustrations on human immunodeficiency virus infections and acquired immunodeficiency syndrome and renal diseases. In his preface to the second edition the author states that "nineteen new illustrations of diseases published in the first edition have been added to broaden the spectrum of clinical presentation of these entities."

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Imaging of the Head and Neck

Galdino E. Valvassori, Mahmood F. Mafee, and Barbara L. Carter. Hard cover, illustrated, indexed, 548 pages, 1995. Thieme Medical Publishers, New York, NY, $199.

This book follows *Head and Neck Imaging*, by basically the same authors, written in 1988. New chapters include the one on the orbit, mandible, and applications of ultrasound and diagnosing head and neck pathology. This is not to say that the 1988 volume is now obsolete, because I do not think that there are any monumental changes in imaging of the head and neck, especially in contrast to the authors' 1982 publication, which was titled *Radiology of the Head and Neck*, a book that became obsolete because of the exponential changes that occurred in computerization and development of nonionizing magnetic resonance techniques.

Magnetic resonance imaging (MRI) continues to be more instrumental in diagnosing lesions of the cochlear and vestibular systems, though this seems to be more for images shown at meetings, rather than in everyday clinical practice, where these types of lesions do not so cleverly show up in these studies. An example is the "lighting-up effect" produced by a viral lesion in the fluid of the labyrinthine system. Of interest, MRI is the choice of study (rather than computed tomography [CT]) in vestibular aqueduct and endolymphatic sac assessments. Also, if one excludes the carotid artery, magnetic resonance angiography seems not to be that important in routine clinical practice at this time. This technology will change, surely, in the near future.

Most images shown in this book are of extremely high quality. Many times, T1- and T2-weighted images are included in the illustrations. I like the fact that these authors have used numbers, letters, and arrows to identify structures, rather than bridge lines with label structures and names, which is so often done in such textbooks. Cadaveric sectioned specimens and line drawings are very helpful, as demonstrated in this text. Conventional radiography has not been neglected in this book, and obviously, conventional radiographs remain the starting point of many imaging studies. The skull base material is very brief.

The practice of head and neck medicine has been profoundly changed by imaging techniques, and this book, more so than many similar ones, shows how imaging has been made more relevant and more exciting. Prior to CT, there was no way to demonstrate the soft tissue depth and infiltration of a head and neck lesion, such as a nasopharyngeal carcinoma. Sound knowledge of CT and MRI is a prerequisite of head and neck medicine, and this book does much to provide some of this knowledge. It is recommended to all otolaryngologists, but mainly to those who have not read the previous volume from 1988.

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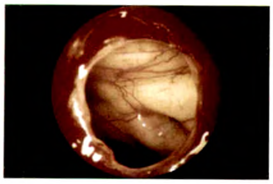

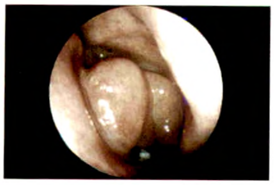

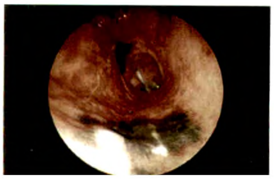

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ANNALS OF OTOLOGY, RHINOLOGY & LARYNGOLOGY (ISSN 0003-4894) is published monthly by Annals Publishing Company, 4507 Laclede Avenue, St Louis, MO 63108. Second-class postage paid at St Louis, Mo. POSTMASTER: Send address changes to *ANNALS OF OTOLOGY, RHINOLOGY & LARYNGOLOGY*, c/o Annals Publishing Company, 4507 Laclede Avenue, St Louis, MO 63108.

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ANNALS OF OTOLOGY, RHINOLOGY & LARYNGOLOGY

VOLUME 105 NOVEMBER 1996 NUMBER 11

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MANAGEMENT OF CERVICAL METASTASES IN SUPRAGLOTTIC CANCER

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A retrospective review of patients from 1979 to 1988 was performed to assess the efficacy of neck dissection, prognostic factors, and the philosophy of treatment of the neck in supraglottic cancer. Of the 89 patients available for analysis, 26 were managed by horizontal partial laryngectomy (HPL), 44 by primary radiotherapy (RT), and 19 by total laryngectomy (TL). A total of 41 patients from the group had 63 neck dissections (NDs); 22 had bilateral and 19 unilateral dissections. A correlation of the pN with N staging revealed that when presenting with N2a nodes (>3 cm), one third had contralateral metastases, and with N2b (multiple), 100% had contralateral metastases. In multivariate analysis of the disease-free interval, age and staging emerged as independent prognostic variables. Although we observed no increased morbidity by dissecting the opposite side, our results did not support routine bilateral neck dissection in N0 patients. However, when the nodes are larger than 3 cm, or ipsilateral and multiple, bilateral neck dissection is recommended.

KEY WORDS — lymph nodes, neck dissection, partial laryngectomy, radiation, supragottic laryngeal cancer.

INTRODUCTION

The management of squamous cell carcinoma of the supraglottic larynx requires a different philosophy than that of glottic tumors; because of the tissues involved and the natural areas of spread of supraglottic tumors, the lymphatic system is involved at a much earlier phase in the disease, and thus, neck nodal metastases are much more common. The main question that remains is whether or not, in the patients in whom clinical metastases are not apparent, steps should be taken to eradicate occult metastases. If the decision is made to do this, should it be by surgery or by irradiation, and should it be only on the side of the lesion or on both sides?

Supraglottic cancer is defined as a lesion affecting the area above the true vocal cords, and includes the roof and lateral walls of the sinus of Morgagni, the false vocal cords, the aryepiglottic folds, and the laryngeal side of the epiglottis. Invasion may take place in the preepiglottic space, the base of the tongue, and the prelaryngeal strap muscles, as well as the pharynx, usually the pyriform fossa.[1] These areas all have a rich lymphatic supply, accounting for the fact that neck metastases are so frequent.[2] Although an examination of the barriers and compartments involved shows that cancer may easily spread from the supraglottis,[3] in certain patients local excision of the supraglottic area by horizontal partial laryngectomy (HPL) has been documented as being oncologically sound.[4,5]

The remaining dilemma is the question of the initial treatment of the neck. Given the fact that nodal metastases are common, many may be in the occult (not clinically detectable) phase at initial presentation. Also, the nature of supraglottic disease is such that it must often be considered as a midline lesion, so that potentially bilateral occult nodes may exist. It has been suggested that for this reason, bilateral routine neck dissection should be performed,[6] or that the neck should be irradiated electively in all patients.[7]

In an attempt to resolve this impasse, we decided to evaluate our most recent patients for whom 5-year statistics were available. End points in this retrospective study were to assess how the presence of ipsilateral and contralateral neck metastases influenced the outcome of treatment in this group of patients, and what the effectiveness was of surgery in preventing relapse. Particularly, we wanted to define the most significant prognostic factors in relation to disease-free interval, and finally, to use this information to ascertain what the approach to treatment of the neck in supraglottic cancer should be.

PATIENTS AND METHODS

A retrospective review was performed of the clinical course of all patients presenting with supraglottic

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Presented at the meeting of the American Laryngological Association, Palm Desert, California, April 29-30, 1995.

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TABLE 1. P VALUES FROM PROPORTIONAL HAZARD (LIKELIHOOD RATIO TEST) ANALYSIS

Variables	*Regional Recurrence*	*Metastases (Univariate)*	*Disease-Free (Univariate)*	*Disease-Free (Multivariate)*
Age	.14		.015	.0005
T classification	.13	.25	.041	.37
N classification	.0056	.0001	.0012	.19
Staging			.0003	<.0001
pT classification	.74	.88	.31	.60
pN classification		.043	.043	.11
p stage			.033	.39
No. positive nodes	.44			
Bilateral neck dissection	.24		.71	.051
Transcapsular spread	.93		.12	.12

laryngeal cancer over the 10-year period from 1979 to 1988. There were 105 patients seen; 11 were excluded because they were not seen after their initial presentation. This left an evaluable group of 94 patients, of whom 26 were managed by (supraglottic) HPL, 44 by primary radiotherapy (RT), and 19 by total laryngectomy (TL). Five patients for various reasons received no treatment at all, so that the analysis was performed on the 89 treated patients.

The following variables were submitted to univariate and multivariate analysis of the disease-free interval: sex, age, unilateral versus bilateral primary tumors, T and N classification, staging (UICC),[8] and the levels of the nodes.[9] We noted the pathologic T and N classifications, the number of positive nodes, the existence of bilateral positive nodes, the features of transcapsular nodal spread, whether or not the neck dissections were radical, and whether or not the patients had bilateral neck dissections. The p values were calculated from the proportional hazard mode (likelihood ratio[LR] test). Interval and ordinal variables were assumed to be linearly related to LR (hazard).

The patients who had neck dissections had their specimens submitted to histologic examination and pathologic (pN) staging, and thus the N and pN classifications could be compared and correlated. Other histologic parameters entered into the analysis were the number of positive nodes and bilateral positive nodes, the presence or absence of transcapsular spread, and whether the neck dissection had positive margins or not. Another parameter entered was whether or not a bilateral neck dissection was performed.

RESULTS

The overall crude 5-year survival was 51%, and the present vital status is as follows: 33 alive without disease, 19 dead of disease, and 37 dead of other causes, ie, intercurrent disease (23) and second primaries (14). The disease-specific 5-year survival was 73% overall, 81% for the HPL group, 75% for the RT group, and 67% for the TL group. The results of the different treatment modalities have been reported elsewhere.[5] Observed regional recurrence rates at 5 years were lower for the surgically treated patients

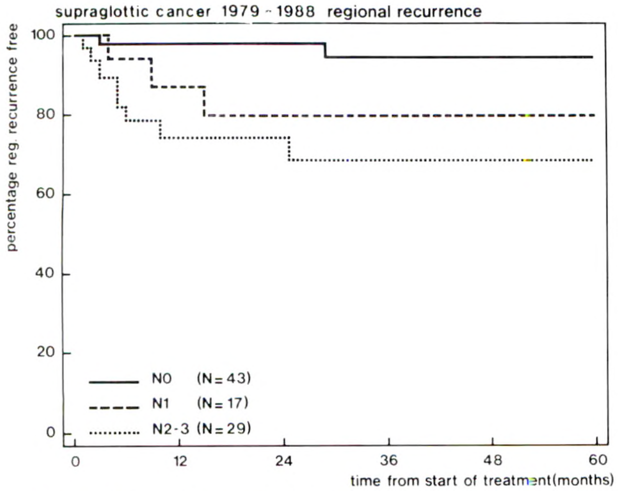

Fig 1. Five-year cumulative regional (reg.) recurrence versus N staging, 1979 to 1988. By univariate analysis, this was highly significant (p = .0056).

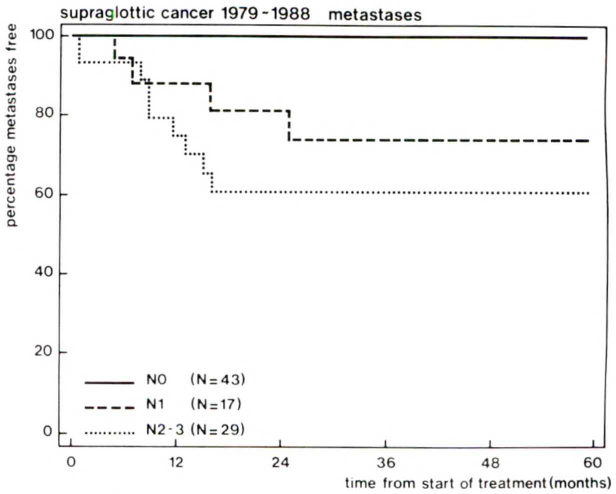

Fig 2. Five-year cumulative rate of distant metastases versus N staging, 1979 to 1988. By univariate analysis, this was highly significant (p = .0001).

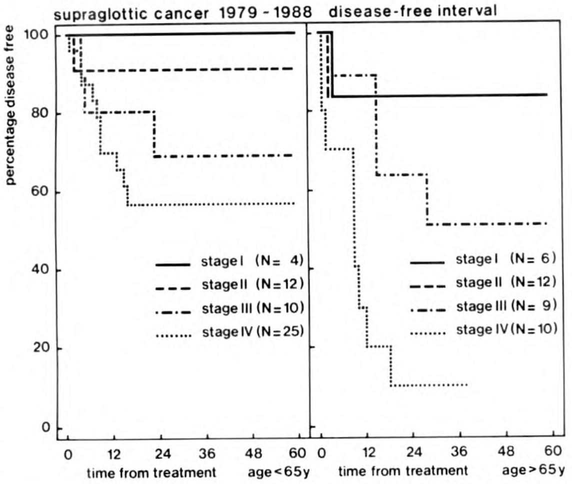

Fig 3. Five-year disease-free interval curves for patients <65 and >65 years old. By multivariate analysis, age and staging emerged as independent prognostic variables.

(TL 11.2%, HPL 14%) and higher for the RT patients (21.3%), but these differences may well be due to chance.

Prognostic Factors Regarding Disease-Free Interval. The results of the univariate and multivariate analyses were as follows (Table 1). Univariately, there is evidence of a relationship between disease-free interval and age ($p \leq .015$), T stage ($p \leq .04$), N stage ($p = .0012$), and staging ($p < .0003$). However, after stepwise analysis, only age ($p = .0005$) and staging ($p < .0001$) emerged as independent prognostic variables. The presence or absence of nodal metastases at presentation is strongly related to the outcome of the treatment in terms of regional recurrence (Fig 1, $p = .0056$) and of distant metastases (Fig 2, $p = .0005$). Not one of the 43 N0 patients developed distant metastases. Figure 3 shows the relationship between disease-free interval and two variables (age and staging) combined. It is clear from this that the patients of 65 years and older did much worse in terms of disease eradication, as did patients in higher stages.

Neck Dissection Related to N Classification. N classification, T classification, and staging for the 89 patients are shown in Fig 4, where it is seen that 43 patients were N0 and 46 had clinical nodes enlarged at presentation. Of these, most patients had nodes in level II (40), and only 16 had nodes in levels III, 3 in level V, and 1 in level VI.[9] Table 2 and Fig 5 show the relationship between the side of the neck nodes clinically and the side of the neck dissections. A total of 41 patients from the group had 63 sides of their necks dissected. Nineteen patients had unilateral neck dissections (10 left and 9 right), and 22 had bilateral neck dissections. (Figure 5A shows the breakdown of these related to the side of the clinical nodes.) Of the 41 patients who had neck dissections, 30 had positive nodes and 11 negative (Fig 5B). Seven N0 patients had an elective neck dissection, of whom only 1 had positive nodes. Of the 46 patients with clinical nodes, 34 had therapeutic neck dissections, of which only 5 were negative. Ten patients with only clinically apparent unilateral nodes (Fig 5A) had had bilateral and therefore contralateral elective neck dissections. Nine of these 10 were positive for cancer contralaterally. Of the 14 patients with clinically bilaterally apparent nodes, 8 had bilateral neck dissections, of whom 6 had histologically positive nodes bilaterally, 1 had positive nodes on one side only, and the other had no positive nodes. Six of these 14 patients received only RT because they were in extremely poor condition.[5]

Nodal Metastases Related to Primary Tumor. Table 3 shows the relationship of *side* of the primary to clinical nodal metastases. Tumors that were centrally located and involved both sides of the supraglottis were classified as "bilateral," and those that only involved one side of the supraglottis, eg, one aryepiglottic fold, were classified as left- or right-sided. In the one-sided primaries (19 left and 13 right), there were no contralateral nodal metastases. With the bilateral primary tumors, however, the incidence of bilateral nodal metastases was nearly 25% (14 of 57 patients). This is reflected in the clinical attitude of the clinicians by the fact that only 1 patient with a

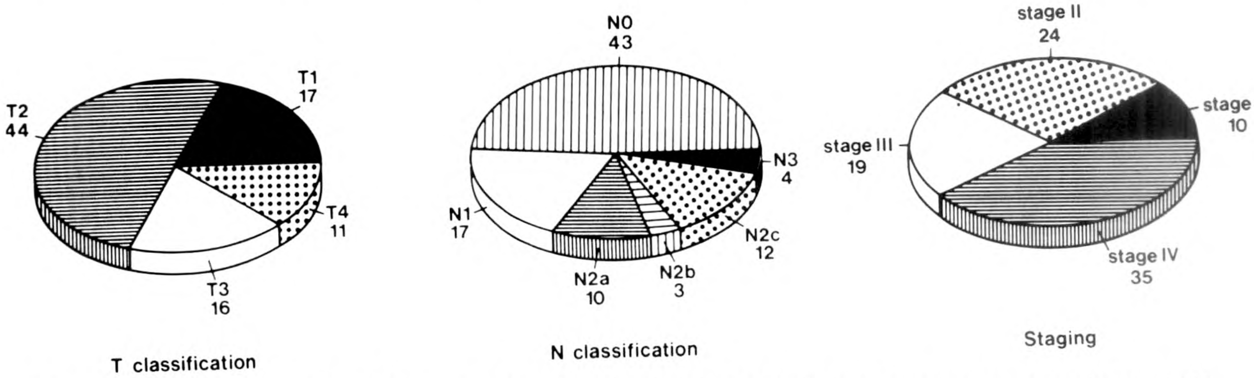

Fig 4. Pie diagrams showing T and N classification and staging for 89 supraglottic larynx carcinoma patients, 1979 to 1988.

TABLE 2. NECK DISSECTIONS PERFORMED IN RELATION TO CLINICAL NODES DETECTED

Clinical Nodal Status	*No. of Pts*	*Pts Not Dissected*	*Left Sides Dissected*	*Right Sides Dissected*	*Total Sides Dissected*	*Bilateral Dissections (No. of Pts)*
N0	43	36	6	5	11	4
Left	16	4	12	4	16	4
Right	16	2	6	14	20	6
Bilateral	14	6	8	8	16	8
Total	89	48	32	31	63	22

unilateral primary had a contralateral node dissection. Table 4 shows, however, that there is no clear correlation between T and N staging.

Relationship Between Preoperative N and Postoperative pN Classifications. The preoperative classification was compared to the pathology results of the 63 specimens in the 41 neck-dissected patients. Nearly one third of N1 patients (n = 16) had contralateral positive nodes. When the presenting nodes were larger than 3 cm (N2a, n = 6), one third had contralateral positive nodes. When the ipsilateral nodes were multiple (N2b, n = 2), 100% had contralateral metastases. Only 14.3% of N0 necks were upstaged to pN1. There were some clinical false positives in the patients with bilateral nodes (n = 8): 12.5% were actually N0 and 12.5% were only unilaterally involved.

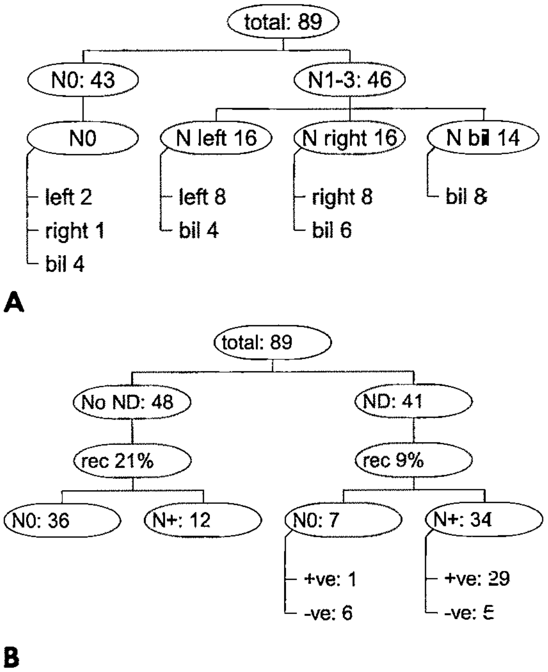

Fig 5. Organizational charts showing relationship of neck dissections to side of clinical nodes. A) Relationship of neck dissections performed to nodal status (in oval zones); figures below refer to patient numbers and neck dissections they had (eg, N left 16 — 16 patients with left-sided nodes). bil — bilateral. B) Neck node status in neck-dissected (ND) and non–neck-dissected patients (No ND), showing 5-year regional recurrence (rec) rates and clinical nodal status (in oval zones) and histologic status below (eg, +ve — histologically positive).

Regional Control. There were 12 regional recurrences among the 89 patients: 3 of 41 from the patients who received neck dissections (5-year cumulative recurrence rate 9%) and 9 of 48 from the patients who did not receive neck dissections, having received RT alone (recurrence rate 21%). This difference may still be due to chance (p = .11). Of the first group, 2 patients received supraglottic laryngectomies: 1 of these had a unilateral and the other a bilateral neck dissection. The latter patient had a recurrence on the left, where an extensive radical neck dissection including skin was performed. The other patient had a recurrence on the opposite side. Both these patients died of distant metastases. The third patient also had a recurrence on the opposite side. This patient was treated by TL and right radical neck dissection and developed nodes on the left soon after treatment. Salvage left neck dissection failed, and the patient died of persistent regional disease with superior vena cava syndrome. In summary, of the neck-dissected group, there were 3 recurrences, of which 2 were on the unoperated side. Neck dissection failed in only 1 of 41 cases to eradicate neck disease.

Postoperative Irradiation. The indication for postoperative RT was based on the following criteria: positive margins in either the primary or the neck dissection; 2 or more positive nodes; or transcapsular spread in the nodes. Of the surgically treated patients, 24 of 45 (12 HPL and 12 TL) received postoperative RT because they fulfilled the above criteria for this. Of the 24 patients, 8 had positive margins of the primary lesion, and 20 fulfilled regional spread criteria. For none of the pathologic variables tested — positive margins of primary, bilateral primary, number of positive nodes, bilateral positive nodes, transcapsular spread, or positive margins of the neck dissection — did we find evidence that they might be independent prognosticators for disease-free interval.

DISCUSSION

As expected with supraglottic cancer, most patients died of causes other than their supraglottic can-

TABLE 3. RELATIONSHIP OF PRIMARY TUMOR AND NODAL METASTASES AS CLINICALLY ASSESSED

Primary Tumor	*N0*	*Left Nodes*	*Right Nodes*	*Bilateral Nodes*	*Total*
Left	11	8	0	0	19
Right	10	0	3	0	13
Bilateral	22	8	13	14	57
Total	43	16	16	14	89

TABLE 4. RELATIONSHIP OF T AND N CLASSIFICATIONS

	N0	*N1*	*N2-3*	*Total*
T1	10	1	6	17
T2	24	6	14	44
T3-4	8	10	9	27
Total	43	17	29	89

cer. Therefore, analyzing the relationship between neck metastases and control of the disease, one needs to look at the disease-free interval or disease-specific survival. Multivariate analysis of the disease-free interval revealed only age and staging as highly significant factors. Although the N classification did not remain significant in multivariate analysis, staging did, and this essentially refers to the presence of nodal metastases. When performing univariate analysis on the prognostic factors for regional recurrence,the N classification again emerged as highly significant; this was also the case when looking at distant metastases (Figs 1 and 2). Ninety-five percent of the N0 patients remained without regional recurrences, while none developed distant metastases. This study has therefore confirmed the findings of others[10,11] that the presence or absence of nodes is of preeminent importance in the final outcome of treatment; this is true for supraglottic cancer as well as for many other head and neck cancers.

Neck dissection emerged as an effective means of eradicating nodal metastases. The regional recurrence rate in the operated patients was 9%: only 3 patients of the 41 neck-dissected patients developed regional recurrence, as opposed to 9 of the 48 patients who were irradiated. Whether or not elective neck dissection was warranted is shown by the fact that in the N0 patients only 1 patient out of 7 (1 of 11 sides) was histologically positive. It suggests that in N0, elective bilateral neck dissection is not required or even justified. This is also borne out by the fact that N0 patients had a low rate of regional recurrence, no matter how they were treated (Fig 1). The implication of the low rate of regional recurrence in the operated patients is that in this series correct judgments were made regarding the need for neck dissection. In the elective neck dissections done for second sides, 9 of 10 were positive. The analysis of the preoperative N classification and postoperative pN classification shows that the chances of opposite disease increase with the N classification, so that when multiple nodes were present, the chance was 100%. The conclusion must be that the correct assessment of the nodal status of patients initially presenting is the single most important event in making treatment decisions. Accurate clinical and imaging techniques[12-14] must still be improved to harvest the full potential of regional management. Routine bilateral neck dissection, however, appears unjustified, and in our series would have resulted in many unnecessary operations. Our regional failure rate of 9% is the same as reported by Weber et al[6] with the use of bilateral neck dissection. When we bear in mind, however, that only 1 patient had a failed neck dissection, the other 2 having recurred on the opposite side, then the recurrence rate for neck dissection becomes 1 in 41, showing that neck dissection, complemented by RT where indicated, is an extremely effective method of eradicating neck disease. Ignoring the failures on the opposite (unoperated) neck makes it impossible to compare the recurrence rate with the patients who did not receive a neck dissection. The other 2 patients might in retrospect have benefited from a bilateral neck dissection (although 1 of them died of distant metastases). In addition to the nodal status of the patient, the side of the primary was also important. In unilateral lesions, there were no nodes on the opposite side (Table 2). In only 1 case did a patient have a neck dissection on the side opposite to a unilateral lesion, reflecting the clinical judgment of the operator. Therefore, 1) the initial examination by the otolaryngologist, assessing the size and side of the primary tumor, 2) the finding of lymph node metastases, and 3) the general condition of the patient are all crucial to the treatment decisions and the eventual outcome.

The type of neck dissection that is adequate to control the disease relates to the usual pattern of spread. In keeping with previous studies,[15] we found that by far the most common site was level II (subdigastric site), with levels I and V being rare. Modified neck dissections, and in some cases selective neck dissections, are therefore justified, particularly on the second side, where sparing of the jugular vein is essential. Of this series, 18 patients had modified radical neck dissections, and 4 patients selective neck dissections, with the remaining 19 having at least one radical neck dissection. The results appear to justify this conservative approach where it is indicated.

It was of some surprise and interest to us that we did not find a prognostic value for the presence of extracapsular spread, the number of positive nodes, or the presence of positive margins in the neck dissections in regional recurrence and disease-free interval. Only for the presence of bilateral nodes was there weak evidence on univariate analysis (p = .07). However, when it is borne in mind that all these features are also criteria for postoperative RT, this may indicate the effectiveness of this treatment, in agreement with the findings of others.[11] Apart from staging, the only other multivariate independent prognosticator was age, patients of 65 and older doing much worse than those under 65. Figure 3 shows the combination of these two highly significant variables. There was a significant difference in age between the surgically treated patients and those primarily irradiated. Several of the irradiated patients were above 65 and generally in very poor condition, with the result that some died before completing their course of RT. There does not appear to be any clear solution for improving the results in these patients.

CONCLUSIONS

The rate of neck metastases was slightly over 50% in this sample of supraglottic carcinoma. The presence of neck metastases (staging) was, in association with age, the only independent prognosticator of disease-free interval. Neck dissection, with postoperative RT where indicated, was an effective method of eradicating regional disease, and in our series achieved a regional control of over 90%, only 1 of 41 neck dissections failing to control regional metastases. Contralateral metastases were related to the primary tumor and to the staging of the ipsilateral neck; therefore, bilateral neck dissection is recommended in all patients with multiple or >3-cm nodes, particularly in combination with midline or bilateral primary lesions. In patients who remain N0 after exhaustive imaging and/or fine needle aspiration cytology examinations, neck dissection need not be performed, and the patients are probably equally effectively treated by RT or by surgery. Postoperative RT appears to be effective in reducing the relapse rate after neck dissection with advanced disease.

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MUSCLE TENSION DYSPHONIA AND SPASMODIC DYSPHONIA: THE ROLE OF MANUAL LARYNGEAL TENSION REDUCTION IN DIAGNOSIS AND MANAGEMENT

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Excessive activity of the extralaryngeal muscles affects laryngeal function and contributes to a spectrum of interrelated symptoms and syndromes including muscle tension dysphonia and spasmodic dysphonia. Recognition of the role of extralaryngeal tension is helpful in ensuring proper diagnosis and selection of appropriate treatment. This report demonstrates the application of manual laryngeal musculoskeletal tension reduction techniques in the diagnosis and management of laryngeal hyperfunction syndromes. The manual technique consists of focal palpation to determine 1) extent of laryngeal elevation, 2) focal tenderness, 3) voice effect of applying downward pressure over the superior border of the thyroid lamina, and 4) extent of sustained voice improvement following circumlaryngeal massage. The clinical utility of this innovative approach is discussed.

KEY WORDS — circumlaryngeal massage, musculoskeletal tension dysphonia, spasmodic dysphonia.

INTRODUCTION

Excessive or "imbalanced" activity of the intrinsic and extrinsic laryngeal muscles affects phonatory function and is considered an important etiologic factor contributing to a spectrum of interrelated symptoms and syndromes including muscle tension dysphonia (MTD) and spasmodic dysphonia (SD).[1,2] Recognition of the contribution of laryngeal and paralaryngeal hypertonicity is critical to proper diagnosis and selection of suitable treatments, regardless of the presumed cause of the voice disorder.

What, precisely, constitutes MTD is unclear. Certain sources have described it as a constellation of vocal symptoms combined with specific glottic and supraglottic contraction patterns.[3-6] This description is contrasted with that of others, who suggest that whereas certain laryngoscopic patterns may be suggestive of excess muscle tension, no particular voice quality or glottic configuration should be uniquely identified with musculoskeletal tension voice disorders.[1] Although confusion surrounds how the disorder should be conceptualized, a recurrent feature in all descriptions is the presence of laryngeal and/or extralaryngeal hyperfunction.[7]

This excessive muscle activity has been attributed to many sources, including 1) psychological and/or personality factors that tend to induce tension,[1,8] 2) technical misuses of the vocal mechanism in the context of extraordinary voice demands,[3-5] 3) learned adaptations following an upper respiratory tract infection,[6] and 4) compensation for underlying disease. Clinically, patients with vocal fold mucosal disease or paralysis or paresis often demonstrate coexisting muscle tension symptoms or patterns. In cases of paralysis, coexisting tension appears to be compensatory, with the patient recruiting extrinsic laryngeal muscles to assist in glottic closure. However, in cases of benign disorders, it is unclear whether the muscle tension pattern temporally preceded the development of the mucosal disease or whether the pattern represents adjustments to compensate for glottic insufficiency. Thus, it is difficult to ascertain whether such muscle tension patterns deserve causal, concomitant, or outcome status. Regardless of the origin, patients with musculoskeletal tension voice disorders usually complain of neck and shoulder tightness, excess vocal effort, and fatigue, with symptoms intensifying with extended voice use. Voice quality symptoms can vary in severity and type, ranging from severely pressed to extreme breathiness with myriad combinations.[1-6]

Spasmodic dysphonia has come to be regarded as a focal laryngeal dystonia.[9-12] Others have described it as a symptom complex with multiple causes, both neurological and psychological.[1,13,14] Despite efforts to improve assessment techniques, the diagnosis continues to be based on auditory-perceptual assessment

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Presented at the meeting of the American Laryngological Association, Orlando, Florida, May 4-5, 1996.

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of vocal symptoms, and to be corroborated by voice therapy failure. As with most dysphonias, SD represents a composite of many elements. Excessive extralaryngeal tension superimposed on the underlying dystonic symptoms can complicate the clinical presentation. This diagnostic predicament is further compounded by the capacity of musculoskeletal tension voice disorders to masquerade as SD.[1,13] Therefore, accurate diagnosis and optimal management of these disorders can be accomplished only be defining the contribution of generalized hyperfunction to the dysphonia. Removing the effects of excessive tension or muscle imbalance affords the clinician a more precise representation of the voice disorder.

In this regard, numerous voice-facilitating techniques are designed to relieve tension in the laryngeal region.[15] Unfortunately, little objective evidence exists to support the superiority of one technique over another, making it difficult to judge the cause of voice therapy failure.[16] Failure may be due, at least in part, to techniques that do not yield sufficient laryngeal tension reduction. Recognizing this, Aronson[1] described and advocated the manual laryngeal musculoskeletal tension reduction technique (also known as circumlaryngeal massage) as a *direct* method to assess and treat laryngeal hyperfunction syndromes. He suggested that chronic posturing of the larynx in an elevated position leads to cramping and stiffness of the hyoid-laryngeal musculature, which contributes to the cause of many dysphonias. He submitted that indirect tension reduction techniques often fail because of the stubborn nature of excess laryngeal musculoskeletal tension. Manual repositioning (lowering) of the larynx by kneading the circumlaryngeal area was offered as the primary approach to reduce tension. Our clinical and research experience[17] with this and related manual techniques has confirmed their clinical utility as valuable diagnostic and treatment tools. This paper describes the use of manual laryngeal techniques in the identification and management of laryngeal hyperfunction syndromes.

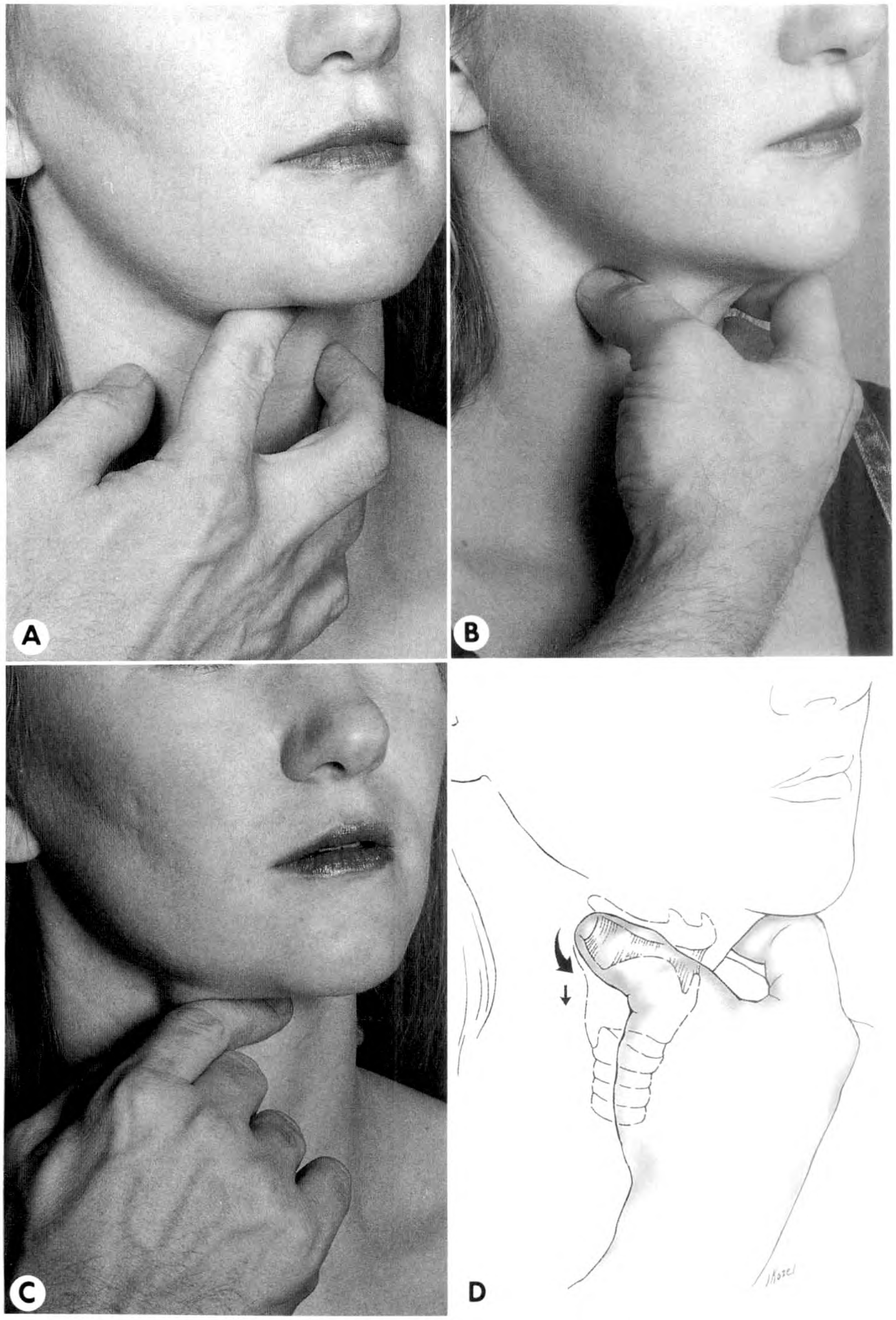
METHODS

Techniques for Diagnosis and Prognosis. When using manual circumlaryngeal assessment and treatment techniques, the patient should be educated regarding the untoward effects of excessive musculoskeletal tension on voice, and the possibility that such tensions may underlie some or all of the patient's dysphonia. The sensitivity of the larynx to stress, emotion, and other sources of tension should also be addressed. If, during the interview, additional insights regarding causation are uncovered, a more complete discussion of the voice-anxiety-tension relationship is appropriate. The patient should appreciate that voice disorders are an aggregate of multiple factors, including potential psychological influences. This understanding is important to maintaining normal voice following successful management. Adjunctive psychological counseling and support may also be indicated.

In most cases in which excess laryngeal musculoskeletal tension has persisted for some time, the larynx is suspended high in the neck and the hyoid-laryngeal sling is extremely stiff. Patients commonly report excess effort expended to produce phonation, as well as a dull to severe ache and tightness of the laryngeal region and anterior neck. Clinicians can assess the contribution of excess tension by evaluating the consequences of tension reduction and laryngeal repositioning on voice quality. The degree of voice improvement appears related to the amount of tension reduction. Reduced pain, reduced focal tenderness or nodularity, increased thyrohyoid space, and improvement in voice confirms the contribution of poorly regulated muscle tension to voice function.

Musculoskeletal tension can be appraised manually by palpation of the circumlaryngeal area to assess the degree, nature, and location of focal tenderness or nodularity (tension) and/or pain in the laryngeal region. Pressure is directed 1) over the major horns of the hyoid bone, 2) over the superior cornu of the thyroid cartilage, and 3) along the anterior border of the sternocleidomastoid muscle and into the suprahyoid musculature, including the posterior belly of the digastric muscle. Focal sites of tension evoke discomfort or pain when pressure is applied. Patients may wince or withdraw (the "jump" sign) when trigger points are identified. This exquisite tenderness in response to pressure in the laryngeal region is considered abnormal. The discomfort is more often unilateral than bilateral, and may radiate to one or both ears. The mobility of the larynx is also tested by attempting to maneuver it side-to-side along the horizontal plane. Resistance of movement is indicative of generalized extralaryngeal hypertonicity. Excessive tension can also be detected in the suprahyoid region (ie, mylohyoid, geniohyoid, and anterior digastric), especially during upward pitch gliding (see Figure, A).

The extent of laryngeal elevation is assayed by palpating within the thyrohyoid space from the posterior border of the hyoid bone to the thyroid notch. A narrowed or absent thyrohyoid space is highly suggestive of excess muscle tension whereby the larynx is suspended high in the neck. In some patients asked to sustain a vowel at a comfortable pitch and loudness, further laryngeal elevation may occur in



Manual laryngeal tension reduction. A) Medial suprahyoid musculature is palpated at rest and during upward pitch glide maneuvers. Focal sites of tenderness and taut bands signal excessive muscle activity. B) Signs of excess laryngeal tension are assayed by 1) evaluating presence of focal sites of pain or nodularity, 2) determining size of thyrohyoid space, and 3) observing voice effect of downward traction over superior border of thyroid lamina and during circumlaryngeal massage. C) Larynx is compressed by exerting anterior-to-posterior pressure over inferior border of hyoid bone. D) Manual tension reduction procedure (circumlaryngeal massage) with hand configuration and placement. Pressure is applied in circular motion over tips of hyoid bone and within thyrohyoid space. Procedure is repeated over posterior borders of thyroid cartilage and larynx is gently pulled downward.

synchrony with voice initiation, signalling recruitment of extrinsic muscles. This type of laryngeal "bobbing" is a frequent concomitant of adductor SD.

Further information regarding inappropriate laryngeal posturing and muscle misuse can be derived by assessing the voice effects of three manipulations: 1) physically impeding elevation of the larynx by applying downward traction over the superior border of the thyroid lamina (see Figure, B); 2) compressing the larynx by exerting anterior-to-posterior (A-P) pressure over the inferior border of the hyoid bone to reduce excess tension and therefore stiffness of the vocal folds (see Figure, C); and 3) applying combined medial compression and downward traction over the superior cornu of the thyroid cartilage.

Laryngeal reposturing or repositioning through brief displacement or by resisting laryngeal elevation provides valuable information regarding potential for improved voice function as well as possible causal mechanisms. By manually stabilizing the larynx, the clinician may stimulate improved voice and briefly interrupt patterns of muscle misuse. These brief moments are immediately identified for the patient and are reinforced. They can be shaped by using digital cueing combined with tension-reduction techniques, to be discussed in the next section. Digital cues can then be faded and the patient taught to rely on sensory feedback (auditory, kinesthetic, and proprioceptive) to maintain improved laryngeal posturing and muscle balance. Manually moving the larynx to a neutral position stimulates easier phonation.

Techniques for Treatment. The manual tension reduction procedure can be undertaken according to the technique of Aronson.[1] The hyoid bone is encircled with the thumb and index finger, which are then worked posteriorly into the tips of the major horns of the hyoid bone. Pressure is applied in a circular motion over the tips of the hyoid bone. The procedure is repeated within the thyrohyoid space, beginning from the thyroid notch and working posteriorly. The posterior borders of the thyroid cartilage medial to the sternocleidomastoid muscles are located and the procedure is repeated there (see Figure, B,D). With the fingers over the superior border of the thyroid cartilage, the larynx is pulled downward and, at times, moved laterally. Sites of focal tenderness, nodularity, or tautness are given more attention. Gentle kneading or sustained pressure may be focused over these sites and then released. In general, the procedure is begun superficially, and the depth of massage is increased according to the degree of tension encountered and the tolerance of the patient. It may be necessary to extend the technique into the medial and lateral suprahyoid musculature. During the above procedures, the patient is asked to sustain vowels or to hum while the clinician notes changes in vocal quality. Improvement in voice and reductions in pain and laryngeal height suggest a relief of tension. The improved voice is shaped from vowels to words, phrases, sentences, and paragraph recitations, and then to conversation. Progress can plateau at any point in the treatment, but recovery takes on its own momentum. Improvements with these techniques often proceed in a nonlinear fashion. Once sufficient tension has been released and the patient assumes a more normal laryngeal posture, progress can be swift, with complete amelioration of the dysphonia. Signs of improvement should be observed within the first session. Some patients may require an extended, intensive treatment session or several sessions, depending on patient tolerance and rate of progress. Generally, if changes do not occur within two sessions, it is unlikely that extralaryngeal muscle tension is the primary or sole explanation for the observed dysphonia. Patients with SD, rather than MTD, may derive short-term benefit from manual techniques; recovery, however, is incomplete and difficult to sustain. The residual dysphonia more accurately reflects the true laryngeal dystonia.

CASE REPORTS

From our series of over 150 cases, the following examples illustrate the use of manual techniques in the assessment and treatment of laryngeal hyperfunction syndromes.

Case 1: Musculoskeletal Tension Dysphonia. A 45-year-old elementary school teacher presented with a year-long history of dysphonia that had begun gradually following upper respiratory tract infection symptoms. Her dysphonia progressively worsened over this period, with no episodes of improved voice. She failed to respond to a 4-week period of voice therapy at a local hospital prior to her referral to our facility. Upon indirect laryngoscopy and videolaryngostroboscopic examination, her larynx was found to be structurally normal, with no evidence of mucosal disease. Noticeable stiffness of both vocal folds was observed, with moderate supraglottic constriction in both the A-P and medial-lateral directions. During a psychosocial interview, the patient denied acute situational conflicts or an increase in life stress preceding the onset of the dysphonia. The patient's vocal quality was described as severely strained-strangled, with frequent voice arrests characteristic of SD. Palpation of the laryngeal region identified focal sites of intense pain over the major horns of the hyoid bone. Although pain was reported bilaterally, it was perceived to be greater on the left side and to refer to the left ear. The larynx was not only sensitive to light pressure, but was also excessively rigid, resisting manual lateral movements. The thyrohyoid space was noticeably narrowed, suggesting laryngeal elevation. Circumlaryngeal massage over the sites of tension and pain was undertaken, and normal voicing was reestablished over a single extended treatment session. The patient reported a relief of tightness and pain concurrent with voice improvements. She was reassessed approximately 1 month later with no evidence of recurrence.

Case 2: Spasmodic Dysphonia. A 35-year-old receptionist reported a 10-month history of gradually deteriorating voice. She complained of increased effort to produce voice, with significant vocal fatigue. She reported feelings of depression, which she attributed to her communication difficulties. Her dysphonia was characterized as pressed-strained, with an abrupt, staccato-like quality consistent with adductor SD. Palpation of the circumlaryngeal area revealed a large thyrohyoid space, with mild tenderness over the superior cornu of the thyroid cartilage. Downward traction on the larynx produced no observable improvement in voice quality. The larynx was mobile laterally. These findings were considered minimal in terms of extralaryngeal tension. A trial of manual laryngeal tension reduction administered over three consecutive sessions was successful in alleviating the tenderness in the laryngeal region. Although modest, transient improvements in voice were detected, they could not be stabilized or generalized. A diagnosis of neurological adductor SD with mild reactive tension was offered. The patient was referred for botulinum toxin injection and responded well. She continues to receive periodic injections with impressive results. She attends a voice disorders support group.

Case 3: Spasmodic Dysphonia With Muscle Tension Dysphonia. A 50-year-old woman had a 4-year history of botulinum injections for SD, with variable but generally favorable results. However, the patient developed an unremitting severe dysphonia (bordering on aphonia) that persisted for 6 months and did not respond to three successive botulinum treatments. A videolaryngostroboscopic examination confirmed the recurrence of hyperkeratotic mucosal changes of the right vocal fold and evidence of posterior laryngitis consistent with laryngopharyngeal reflux. Both vocal folds were extremely stiff and phonation was accompanied by noticeable supraglottic valving. Upon palpation of the paralaryngeal area, the patient complained of significant tightness and pain. Focal sites of nodularity were identified. The thyrohyoid space was minimal. The entire hyoid-laryngeal sling was extremely tense and fixed. Downward traction over the superior cornu of the thyroid cartilage resulted in immediate improvement in voice production. The mucosal changes were judged to be insufficient to explain the severity of the patient's voice disturbance. The patient was considered to have a severe musculoskeletal tension component contributing to her dysphonia. She was submitted to a week-long period of twice-daily therapy sessions, combining multiple voice therapy techniques administered by several clinicians. The voice disorder remained resistant to all indirect tension-reduction techniques. Following intense circumlaryngeal massage, the patient achieved an approximation of normal voicing despite persistent laryngeal mucosal abnormalities. She encountered difficulty in stabilizing these improvements beyond the therapy room. She has been instructed in the self-administration of these manual techniques.

It was speculated that the cause of her extreme laryngeal and extralaryngeal hypertonicity was related to attempts to compensate for both mucosal changes and her underlying dystonic symptoms. These difficulties, combined with concurrent stressful life events and a tendency in this individual for anxiety to manifest in the musculoskeletal system, were sufficient to generate MTD superimposed on mild SD symptoms. In this case, when the deleterious effects of excess muscle tension were stripped from the dysphonia, the clinician was better able to appreciate the consequences of both the vocal fold mucosal changes and the underlying dystonia.

DISCUSSION

Manual techniques, including circumlaryngeal massage, augment the voice practitioner's diagnostic and treatment armamentarium. Given the potential for musculoskeletal tension disorders to coexist with and mimic neurological SD, it is exceedingly important for diagnosticians to appreciate the effects of poorly regulated laryngeal and paralaryngeal tension. Such recognition can avoid unnecessary medical or surgical intervention. These manual techniques can assist in identifying and recognizing the role of laryngeal and extralaryngeal hypertonicity in the perceived voice disturbance. During palpation, the presence of laryngeal pain and tenderness, stiffness, and elevation should raise the index of suspicion for the existence of excessive musculoskeletal tension. The influence of abnormal muscular tensions (ie, nondystonic) can be verified by the patient's positive response to diagnostic therapy using laryngeal reposturing techniques such as circumlaryngeal massage. By identifying neurological SD patients who also present with generalized laryngeal hypertonicity, clinicians can also interpret suboptimal responses to botulinum treatments and prospectively advise a therapy regimen combining manual laryngeal tension reduction (or some other behavioral approach to reduce tension) with botulinum injections.

In summary, our clinical and research experience with this technique leads us to agree with Aronson's[1] contention that all patients with voice disorders, regardless of cause, should be assessed for the presence of excess laryngeal musculoskeletal tension, either as a primary or as a secondary cause of the dysphonia (page 314). Manual laryngeal techniques serve as important procedures in ensuring proper diagnosis of and selection of appropriate treatments for both MTD and SD.

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Ann Otol Rhinol Laryngol 105:1996

ENDOSCOPIC LASER MEDIAL ARYTENOIDECTOMY VERSUS TOTAL ARYTENOIDECTOMY IN THE MANAGEMENT OF BILATERAL VOCAL FOLD PARALYSIS

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Bilateral laryngeal paralysis can result in severe airway compromise. A widely accepted treatment is endoscopic laser total arytenoidectomy (TA); however, vocal results are usually poor. An alternative approach, resection of only the medial portion of the arytenoid (medial arytenoidectomy [MA]), has the potential for less vocal impairment, but may not provide a sufficient airway. Laryngeal resistance (LR) was measured in vivo and ex vivo in 12 adult mongrel dogs with bilateral laryngeal paralysis after TA, MA, or no surgery (controls). The LR was significantly lower than in controls in both TA and MA, but there was no significant difference between TA and MA. Glottic area was also significantly higher in both MA and TA compared to controls, but again, there was no significant difference between TA and MA. Phonation could be elicited in all controls and 2 of 4 dogs with MA, but no dogs with TA. Our results show that MA offers airway improvement similar to that with TA and also has the potential for better vocal function.

KEY WORDS — arytenoidectomy, vocal fold paralysis.

INTRODUCTION

Bilateral vocal fold paralysis usually results in severe airway obstruction and compromise of the laryngeal airway. Prior to 1922, tracheostomy was the only procedure available to the patient with this condition. Since then, unilateral vocal fold lateralization has become the mainstay in treatment. Over the years, a variety of methods have been described to accomplish this task, the goal being to reestablish an adequate laryngeal airway with as little compromise of vocal function as possible.

Woodman[1] in 1946 described extralaryngeal arytenoidectomy using a posterolateral approach. He preserved the vocal process and sutured it to the inferior cornu of the thyroid cartilage. This technique gained wide acceptance and is still widely used today. Thornell[2] in 1948 was the first to describe an endolaryngeal arytenoidectomy. Although there have been excellent results reported from this procedure,[3] it has not been widely accepted because of technical difficulties, bleeding, and unacceptable scarring. Tucker[4] in 1976 introduced a technique of reinnervation intended to restore physiologic motion to the paralyzed larynx. A nerve-muscle pedicle, composed of a small portion of the omohyoid muscle and its branch of the ansa hypoglossi nerve, was transposed onto the posterior cricothyroid muscle. Despite initial reports of good results,[5,6] long-term outcome has been disappointing. Other approaches to reinnervation, such as anastomosis of a phrenic nerve with the recurrent laryngeal nerve stump, have also failed to achieve clinical success. In 1983, Eskew and Bailey[7] reported the use of the carbon dioxide (CO_2) surgical laser to perform endoscopic arytenoidectomy in dogs. In 1984, Ossoff et al[8] described successful clinical use of endoscopic laser arytenoidectomy, achieving restoration of the airway and decannulation in 10 of 11 tracheostomy-dependent patients with bilateral vocal fold paralysis.

In the past decade, CO_2 laser endoscopic arytenoidectomy has become perhaps the most commonly used surgical procedure for enlarging the glottic airway in patients with bilateral vocal fold paralysis. The CO_2 laser permits precision operations through

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Presented at the meeting of the American Laryngological Association, Orlando, Florida, May 4-5, 1996.

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the relatively narrow field of the microlaryngoscope without the need for tissue manipulation.[9] Furthermore, the CO2 laser offers excellent hemostasis.

In 1990, Ossoff et al[9] reported a series of endoscopic laser arytenoidectomies in 28 patients with bilateral vocal fold paralysis. Twenty-six of these had tracheostomies and 2 patients presented with stridor. The airway was successfully restored in 27 patients, although 2 patients required a revision procedure for successful decannulation. The 1 patient that failed had been successfully decannulated after the procedure but developed respiratory distress at 15 months postoperatively and required a tracheostomy. Ossoff et al attributed this to glottic stenosis from scarring secondary to the heat sensitivity of the posterior commissural tissues in addition to subsequent granuloma and posterior glottic web formation.

It has long been accepted that any complete arytenoidectomy for the treatment of bilateral vocal fold paralysis is routinely associated with a poor postoperative phonatory result. All 28 of the patients of Ossoff et al[9] described moderate to severe reductions in phonatory quality postoperatively.

To address the problems of uncontrollable scarring and poor voice quality associated with total arytenoidectomy (TA), Crumley[10] developed endoscopic laser medial arytenoidectomy (MA), a procedure aimed at enhancing the airway with little or no loss of laryngeal phonatory function. He reported a series of endoscopic laser MA in 8 patients: 2 with stridor and 6 with indwelling tracheostomies. Of the 2 patients without tracheostomies, the airway was enhanced and phonatory voices were preserved; 2 of the 6 tracheostomized patients were decannulated. A concern with MA is that it may not resect enough tissue to restore an adequate airway.

This report compares the aerodynamic results of endoscopic laser MA and TA in a series of dogs with surgically created bilateral vocal fold paralysis. We hypothesized that MA and TA would result in a similar decrease in laryngeal resistance, but induced phonation would be less compromised in MA.

METHODS

Twelve adult male mongrel dogs weighing 22 to 32 kg were divided into three groups of 4 dogs each: group 1 was the control (no procedure), group 2 had endoscopic laser TA, and group 3 had endoscopic laser MA. All laser procedures were carried out with a Xanar Coherent 20W CO2 laser with a Unimax model 2000 CO2 Microspot Micromanipulator (Reliant Technologies, Inc, Foster City, Calif) using a spot size ranging from 0.15 to 4.5 mm. The laser was coupled to a Zeiss operating microscope with a 400-mm lens. Each dog was premedicated intramuscularly with acepromazine maleate (0.11 mg/kg). Anesthesia was induced by intravenous sodium pentothal (1.8 to 3.6 mg/kg) and maintained by isofluorane via a CO2 laser protective metal orotracheal tube. All operations were carried out by the primary author to avoid introducing operator variability. Via a ventral midline incision in the neck, the left recurrent laryngeal nerve was identified and a 3- to 4-cm segment of the nerve was excised. The identity of the nerve was confirmed by direct laryngoscopy, observation of vocal fold motion with electrical stimulation of the nerve, and immobility after resection. Group 1 (control) underwent transection of bilateral recurrent laryngeal nerves and then underwent acute physiologic studies. In groups 2 and 3, the incision was closed and endoscopic arytenoidectomy was performed.

Endoscopic CO2 laser surgery was performed with a vaginal speculum used as a suspension laryngoscope. Topical 1% lidocaine was used to decrease postoperative laryngospasm. The arytenoid cartilage, including both the vocal and muscular processes, was totally ablated in group 2 dogs (TA) as described by Ossoff et al.[9] Medial arytenoidectomy as described by Crumley[10] was performed in group 3 dogs, creating an ipsilateral concavity in the arytenoid body. The vocal process, muscular process, and posterior commissure were left intact. Care was taken not to produce laser injury inferior to the arytenoid cartilage, thus preventing injury to the cricoarytenoid joint. The surgery was performed on the left side for all animals.

Physiologic Studies. Approximately 4 weeks were allowed for healing and reepithelialization of the raw surface created by the arytenoidectomy. The animals were brought to the laboratory for physiologic studies. They were premedicated intramuscularly with acepromazine maleate (1.1 mg/kg) and then anesthetized with an intravenous injection of alpha chloralose (0.1 g/kg) and urethane (1.0 g/kg). The animal was placed supine on the operating table and a peripheral vein was cannulated with a 16-gauge catheter so that additional intravenous anesthesia could be administered as needed. Prior to starting the physiologic studies, all animals were studied with videolaryngoscopy. The larynx was visualized with a rigid Karl Storz 0° telescope coupled to a color video camera, with illumination provided by a 150-W light source. The right vocal fold was carefully tattooed with the tips of small forceps stained with a blue permanent ink marker. The length between the points of the forceps was initially measured, as this represented a known distance on the vocal fold. The images of the larynx were recorded on a VHS tape recorder. The

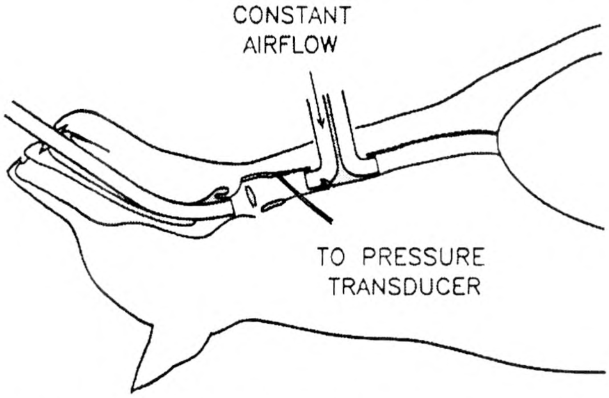

Fig 1. Dog breathes spontaneously through lower tracheal cannula. Constant stream of warm, humidified air is passed into upper tracheal cannula and flows through larynx in expiratory direction. Pressure transducer inserted through cricothyroid membrane measures subglottic pressure.

NIH Image 1.57 software was used to capture the images of the larynx from the videocassette onto a Macintosh personal computer. Several frames were selected for analysis of each subject. The area of the glottic airway was calculated by the software program, with the tattooed points used as a known distance for calibration.

Attention was then turned to the neck area. An anterior longitudinal midline cervical incision was made from the hyoid bone to the sternal notch, and the strap muscles and sternocleidomastoid muscles were retracted laterally to expose the larynx and trachea (Fig 1). A distal tracheostomy was performed at the level just superior to the suprasternal notch, and an endotracheal tube was passed caudally to allow for spontaneous breathing. Attention was turned to the right tracheoesophageal groove, where the intact right recurrent laryngeal nerve was identified and transected, thus creating a bilateral vocal fold paralysis. The trachea was then transected horizontally and a large-bore polyethylene tube, which provided an airtight seal in the trachea, was placed in the rostral segment and directed toward the larynx. This tube

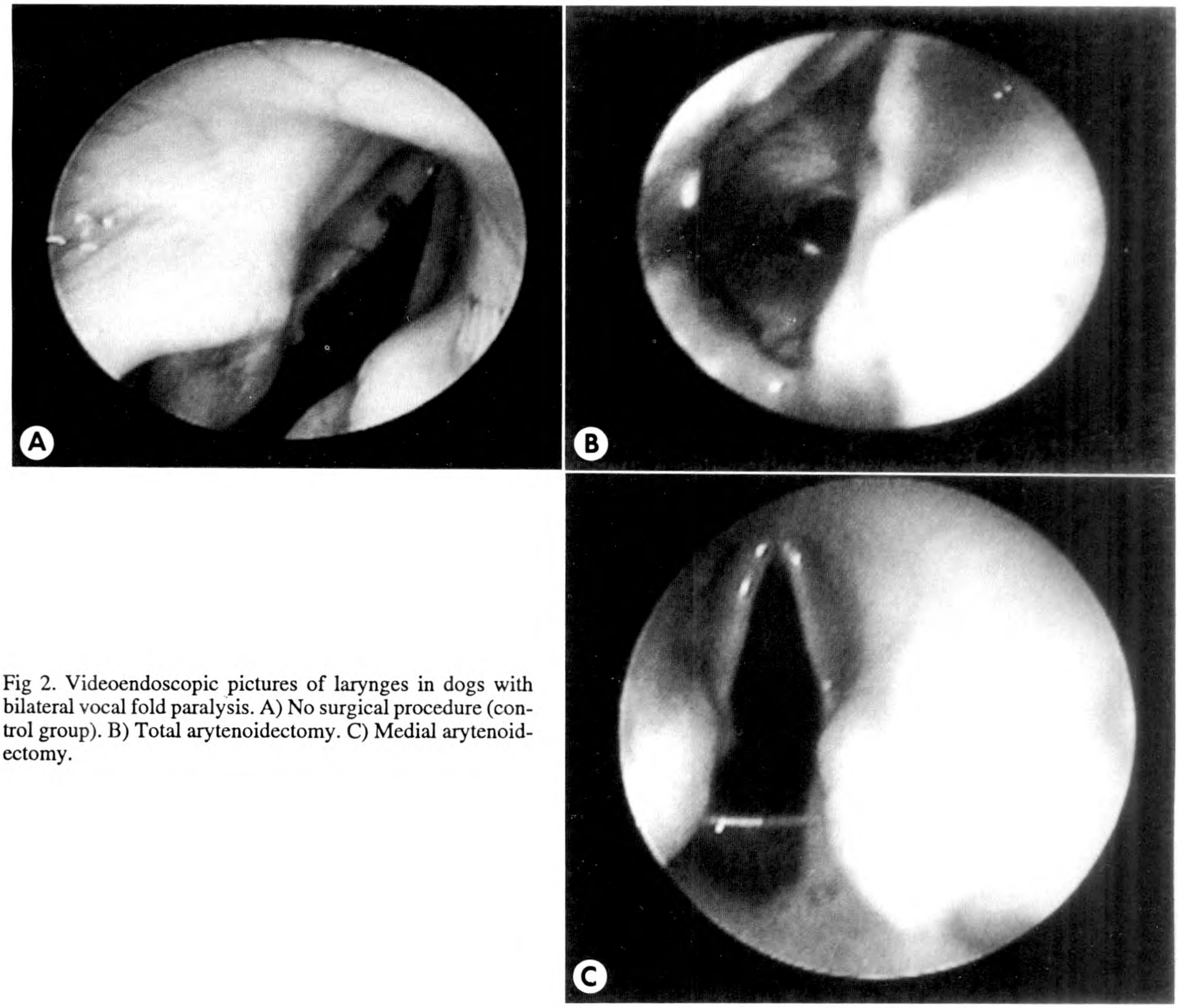

Fig 2. Videoendoscopic pictures of larynges in dogs with bilateral vocal fold paralysis. A) No surgical procedure (control group). B) Total arytenoidectomy. C) Medial arytenoidectomy.

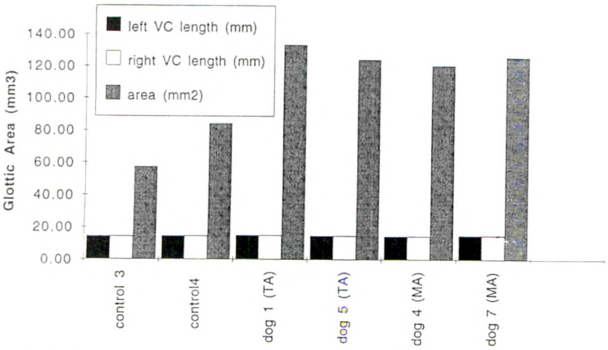

Fig 3. Calculated glottic area in square centimeters for 2 dogs in each group. TA — total arytenoidectomy, MA — medial arytenoidectomy.

was connected to a laboratory wall outlet and used to pass a constant flow of warm, humidified air in an expiratory direction. Airflow was controlled by a valve at the laboratory wall outlet and was humidified and heated by bubbling through 5 cm of heated water so that the temperature of the air was approximately 37°C. The airflow was measured in liters per minute with a Hans Rudolph pneumotachometer. Before recording any trials, the pneumotachometer was calibrated with known levels of airflow from a Manostat Teleflow flowmeter. The output of the pneumotachometer was digitized and later analyzed by a Macintosh personal computer with the Super Scope II software (GW Instruments). A large polyethylene tube was also inserted through the mouth with the tip just below the epiglottis to eliminate fluctuations in resistance above the larynx. A 16-gauge catheter was inserted through the cricothyroid membrane and positioned below the glottis. The catheter was connected to a Spacelab 414 dual pressure Opt 21 pressure transducer and used to measure subglottic pressure. Subglottic pressures were recorded at laryngeal airflows of 20, 40, 60, 100, and 150 L/min. An average of two trials were run for each rate of airflow.

The animals were euthanized and the larynges harvested for ex vivo studies. The ex vivo larynx was securely mounted via six-point suspension in a fiberglass superstructure. Subglottic pressures at the various airflows were measured by the same setup as described above.

Laryngeal resistance was calculated as the ratio between subglottic pressure and the constant airflow through the larynx. Results from two trials at each laryngeal flow rate were averaged for each dog.

RESULTS

Videolaryngoscopy. Figure 2 depicts a representative image of the larynx from each of the three groups.

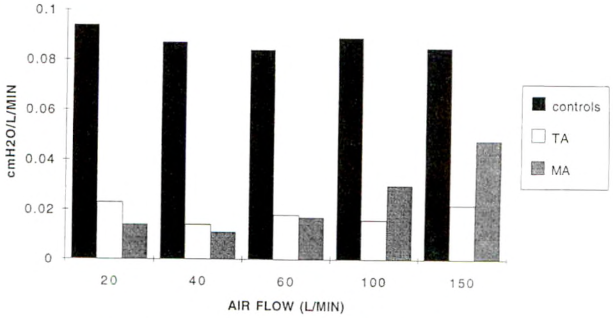

Fig 4. Mean laryngeal resistance (in vivo) in control dogs and dogs that underwent TA and MA.

It was possible to calculate the area of the glottis for only 2 dogs in each group with the NIH Image software. The average area for group 1 was 70.9 mm^3, group 2 was 128.8 mm^3, and group 3 was 123.3 mm^3 (Fig 3). When submitted to unpaired Student's *t*-test, the glottic area was significantly larger than control in both surgical groups ($p < .0005$). There was no statistical difference in glottic area between the two surgical groups.

Laryngeal Resistance. In vivo data are displayed in Fig 4. At all flow rates, laryngeal resistance was significantly higher in control dogs than in either surgical group. At airflows of 60 L/min or less, the mean airway resistance in the MA group was less than or equal to that of the TA group. The difference was not statistically significant on Student's *t*-test. At 100 L/min, in vivo resistance was higher in the MA group, but the difference was not statistically significant ($p = .216$). At 150 L/min, resistance was an average of 0.048 cm H_2O/L per minute in the MA group versus 0.022 cm H_2O/L per minute in the TA group ($p < .0005$).

Figure 5 represents data obtained from aerodynamic studies in excised larynges. There was no statistically significant difference between experimental groups in airway resistance. At airflows of 40 L/min and above, resistance was significantly lower than control values in both experimental groups ($p < .0005$).

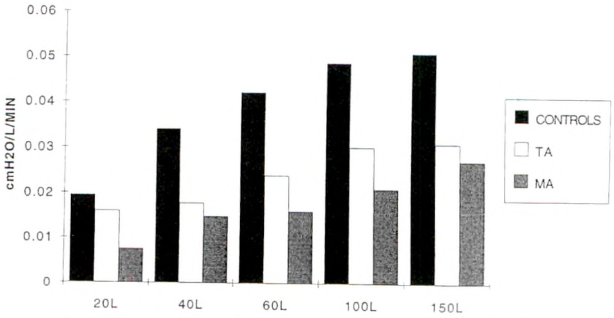

Fig 5. Mean ex vivo laryngeal resistance in control dogs and dogs that underwent TA and MA.

Flow rates of 60 L/min to 100 L/min resulted in phonation in all the control dogs. Phonation did not occur in the TA group, regardless of flow rate. In 2 of the dogs with MA, phonation occurred at rates of 80 and 100 L/min.

DISCUSSION

In this animal model, at physiologic flow rates, MA and TA resulted in similar glottic airway resistance, despite the fact that TA removes a larger volume of cartilage. Also, the two procedures resulted in essentially the same cross-sectional area of the glottis, as determined by image analysis of videoendoscopy. These findings may seem counterintuitive, since TA removes a larger volume of cartilage than is excised in MA. Further, MA preserves the vocal process, the point of attachment of the cartilaginous and membranous vocal folds. It has been suggested that preservation of the vocal process would prevent the membranous vocal fold from retracting laterally, to minimize the anterior glottal gap and conserve vocal function. This should result in a narrower overall glottic opening in MA as opposed to TA. Conversely, transverse cordotomy is intended to enlarge the glottic airway by severing the attachment of the membranous vocal fold to the arytenoid.[11] The results of the present study support the notion that the crucial glottic airway is the posterior, cartilaginous portion.

Another consideration, which is consistent with the results of this study, is that the narrowest part of the upper airway is the cricoid cartilage. This is the rate-limiting segment. The arytenoid cartilage articulates on the rostral surface of the cricoid cartilage, and only a portion extends into the lumen of the airway. Thus, maximal airway enlargement could be achieved by excising that intraluminal portion of the arytenoid cartilage. Further resection, lateral to the medial surface of the cricoid, would not result in any additional increase in effective airway.

An additional factor may be scar contracture. Total arytenoidectomy results in a large soft tissue defect. Cicatrization would be expected to diminish the airway. In MA, the remaining portion of the arytenoid could serve to support the soft tissues and limit contracture.

Phonation could be induced in all control dogs, with bilateral vocal fold paralysis, but in none of the dogs after TA. This is consistent with the clinical observation that TA results in vocal impairment. The MA was devised with the intention of preserving as much vocal function as possible. The findings of this study suggest that this may be achieved, as phonation could be induced in 2 of the dogs in the MA group.

Resistance was tested at a variety of flow rates, since resistance is frequently not linear. In all animals, resistance increased with airflow. The higher rates of flow in this study are considerably outside the physiologic range for normal or labored breathing. Thus, the statistically significant difference noted in airway resistance at 150 L/min between the two experimental groups would not be clinically significant.

It was presumed that activity in extrinsic laryngeal muscles, such as cervical strap muscles, may affect laryngeal resistance. Changes in the rostrocaudal position of the larynx have been reported to affect transglottic resistance.[12] Therefore, we conducted aerodynamic studies in the excised larynx, in addition to the in vivo studies, to control for variability in extrinsic muscle activity in the living preparation, and to study the morphologic effects in isolation. As in the in vivo preparation, laryngeal resistance was significantly lower in both experimental groups compared to the control group.

Clinical application of the results of this study should be tempered by several factors. First, there are differences between dogs and humans in terms of anatomy and healing properties. Second, this animal model does not precisely replicate the clinical presentation. Candidates for arytenoidectomy usually present with chronic bilateral paralysis, and paralyzed vocal folds are commonly observed to move medially over time, so that the glottic airway is narrower than in acute laryngeal paralysis. In this model, due to financial and logistical considerations, surgery was carried out on an acutely paralyzed vocal fold. To diminish the costs of boarding the animals, only 1 month was allowed for healing. In order to avoid the need for a tracheostomy, which is poorly tolerated in dogs, the contralateral vocal fold was not paralyzed until the time of the terminal physiologic studies.

Despite these limitations, the study indicates that MA is promising as an alternative to TA, to restore the airway in patients with bilateral laryngeal paralysis, yet conserve vocal function.

CONCLUSIONS

1. Laryngeal resistance was significantly less in animals that underwent endoscopic laser TA or MA as compared to no vocal fold lateralization procedure.
2. There was no statistically significant difference

between MA and TA in laryngeal resistance at airflows 100 L/min or less.

3. The area of glottic opening was significantly greater in the animals that underwent either TA or MA as compared to no vocal fold lateralization procedure.
4. There was no statistically significant difference in glottic area between TA and MA.

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PEDIATRIC OTORHINOLARYNGOLOGY

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Ann Otol Rhinol Laryngol 105:1996

ROLE OF BRONCHOALVEOLAR LAVAGE IN HOSPITALIZED PEDIATRIC PATIENTS

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Bronchoalveolar lavage (BAL) has been shown to be a rapid, relatively safe, and relatively noninvasive diagnostic procedure. Theoretically, BAL can be performed on all children hospitalized for pneumonia resistant to oral antibiotics, though practically and economically, this is not feasible. A 1-year retrospective review was conducted to define a cost-effective role for BAL in the management of hospitalized children with resistant pneumonia. The data revealed identification of at least one pathogen in 87% of sputum samples and in 95% of BAL specimens. Sputum samples provided the same information as the more invasive BAL technique in 60% of patients who had both sputum and BAL obtained for culture. Recommendations are made for the use of BAL as a diagnostic tool in the hospitalized child with resistant pneumonia.

KEY WORDS — bronchoalveolar lavage, bronchoscopy, immunocompromised child, pneumonia.

INTRODUCTION

Pulmonary processes are a major cause of morbidity and mortality in the chronically ill and immunocompromised pediatric patient.[1-3] Diffuse pulmonary infiltrates seen on radiographic evaluation are a particular diagnostic challenge, as causes may include infections, neoplasms, drug- or radiation-induced toxicity, pulmonary edema, or nonspecific pneumonitis.[2,3]

Routinely, children with pneumonia resistant to oral antimicrobials are admitted to the hospital, and empirically, broad-spectrum intravenous antibiotics are instituted. Coverage is provided to include presumptive organisms much the same as in empiric treatment for sinusitis or pharyngotonsillitis. Although intravenous therapy with broad-spectrum antimicrobials is not without potentially serious risk, the majority of children improve without untoward events.[3] In those who do not improve, a diagnosis must be confirmed. Hence, a useful diagnostic test must be identified.

Noninvasive sputum sampling, be it random or induced, requires the participation of a mature, cooperative child. Bronchoalveolar lavage (BAL) has been shown to be a rapid, relatively safe, and relatively noninvasive diagnostic procedure for the evaluation of diffuse pulmonary infiltrates.[3-9] More invasive techniques, such as open lung biopsy, are also useful in establishing a definitive diagnosis, but are associated with significant morbidity, and as such, are reserved for those patients in whom diagnoses cannot be obtained by less invasive methods.[2,7] Theoretically, BAL may be used for all pediatric patients admitted with the diagnosis of pneumonia resistant to oral antimicrobials, though practically and economically, this is not feasible. The purpose of this study is to determine a cost-effective role for BAL in the management of hospitalized children with resistant pneumonia.

METHODS

A retrospective review was performed on the charts of those children who underwent BAL during the period from April 1, 1992, to March 31, 1993, at The New York Hospital–Cornell Medical Center, a tertiary referral center. Twenty-one patients underwent BAL. Several patients underwent multiple procedures, but for the purposes of this study, only cumulative results are discussed. The patient population consisted of 9 boys and 12 girls. Ages ranged from 3 months to 17 years. All 21 patients were evaluated for diffuse pulmonary infiltrates or interstitial disease that failed to improve or worsened despite antibiotic

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Presented at the meeting of the American Broncho-Esophagological Association, Palm Desert, California, May 2-4, 1995.

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therapy.

Several factors contributed to chronic illness and recurrent hospitalizations for pneumonia in 15 of the 21 patients studied. Ten patients were immunocompromised. Underlying diagnoses included chemotherapy for neoplastic disease (n = 5), steroid therapy (n = 2), human immunodeficiency virus (n = 2), and severe combined (B and T cell) immunodeficiency (n = 1). Five children had anatomic predisposing factors: 4 had gastroesophageal reflux that was documented by pH probe or milk study, and 1 had aberrant pulmonary architecture. One child was at increased risk for tuberculosis as a result of environmental exposure. The remaining 5 patients were hospitalized for pneumonia without documented underlying immunologic or anatomic disorders.

Sputum samples were obtained in 15 of the 21 patients. Sputum samples were usually obtained early in the course of hospitalization. The chart review did not reveal whether the samples were random expectorations or induced sputum specimens. To submit an induced sputum sample, the child first gargled with normal saline to reduce the colonization of normal mouth flora. Next, an ultrasonic DeVilbiss sterile water nebulizer treatment was administered. Finally, the child was instructed to cough and expectorate into a sterile cup. Chest physiotherapy was employed as an adjunctive measure.

Patients who were mechanically ventilated underwent flexible fiberoptic bronchoscopy using the Olympus BF3C20. Examination of the airway to rule out structural abnormalities, as well as to obtain the culture material, was accomplished. Premedication consisted of bolus doses of fentanyl citrate (1 to 2 μg/kg), midazolam maleate or lorazepam (0.05 to 0.10 mg/kg), and vecuronium bromide (0.10 mg/kg). The bronchoscope was passed directly into the endotracheal tube via a Bodai adapter so as to permit concurrent ventilation during the procedure. Electrocardiographic and oxygen saturation measurements were monitored throughout the procedure. The bronchoscope was passed into the trachea, and initially the entire tracheobronchial tree was examined. Next, the area of concern was localized and lavaged with a total of 20 to 40 mL of sterile saline. The specimens were aspirated separately by vacuum suctioning in 3- to 6-mL aliquots and then pooled.

Patients who were on supplemental oxygen by nasal cannula or face mask underwent flexible or rigid bronchoscopy and lavage under general anesthesia. Similarly, the entire tracheobronchial tree was examined. Next, the bronchoscope was placed into the areas of greatest clinical and/or radiographic concern. Lavage was performed in an identical fashion.

Specimen Studies. The pooled lavage fluid was apportioned for the individual laboratories within 3 hours of collection.

In the microbiology laboratory, BAL fluid was centrifuged for 5 minutes at 3,000 rpm. The supernatant was discarded and the sediment was used for Gram's stain in the customary manner. Cultures were incubated and read accordingly.

In the virology laboratory, the pooled lavage fluid was immediately transferred to viral Hanks' balanced salt solution with antibiotics. It was then placed in one of two tissue cell culture lines: human embryonic lung, or primary monkey kidney. It was placed in incubation in cell culture maintenance medium (2% fetal calf serum; Biowhittaker Co, Walkersville, Md) and evaluated after 2 to 3 weeks for cytopathic changes.

Respiratory syncytial virus was evaluated by nasopharyngeal swab and BAL using direct fluorescent monoclonal antibody staining with fluorescein isothiocyanate–labeled mouse monoclonal antibody against respiratory syncytial virus internal capsid protein and envelope surface glycoprotein.[6] Cytomegalovirus (CMV) was detected by cytopathic changes in cell culture. Additionally, a rapid fluorescent evaluation was performed on specimens incubated on coverslips for 48 hours. Finally, some specimens were evaluated by polymerase chain reaction.[8,9]

To evaluate for mycobacteria, a solution of N-acetyl-L-cysteine in sodium hydroxide and sodium citrate solution was added to the specimen and centrifuged for 15 minutes at 3,000 rpm. The supernatant was discarded and the sediment was resuspended in 1 mL of buffer to enhance growth. A Lowenstein, Jensen, and Middlebrook 7H11 plate was inoculated and incubated for 2 weeks in 5.1% carbon dioxide at 35°C with the cap slightly ajar to facilitate evaporation. After 2 weeks, the culture was transferred to a standard incubator, where it remained for 6 weeks. The cultures were surveyed weekly for contamination and growth. Once growth was seen, a Kinnion acid-fast smear was made. A Gen-Probe (Gen-Probe, Inc) was used for rapid identification, and sensitivities were prepared. Additionally, auramine O fluorescent evaluation was performed on the fluid on the day of processing as a preliminary method of screening.

For mycology, the BAL fluid was inoculated on three media: Sabouraud, Mycosel, and inhibitory mold agars. They were incubated for 3 weeks at 30°C and evaluated weekly. A KOH-Calcafluor potassium hydroxide and fluorescent brightener (28 Calca-

TABLE 1. SPUTUM ISOLATES (N = 15)

	RSV	*Bact*	*PMN*	*PCP*	*AFB*	*CMV*	*Fungus*	*Yeast*	*Pert*	*Leg*	*Cand*
Positive	2	9	9	0	2	0	0	0	1	0	4
Negative	10	5	4	7	9	1	3	4	4	5	0
N/A	3	1	2	8	4	14	12	11	10	10	11

Percent yield was 13 of 15 or 87%.

N/A — culture not obtained, RSV — respiratory syncytial virus, Bact — bacteria, PMN — polymorphonuclear cells, PCP — *Pneumocystis carinii*, AFB — acid-fast bacteria, CMV — cytomegalovirus, Pert — *Bordetella pertussis*, Leg — *Legionella*, Cand — *Candida albicans*.

fluorM2R fluorescent stain, Sigma Chemical Co, St Louis, Mo) was also used on the BAL fluid. It was mixed on a coverslip and examined under the microscope.

To evaluate for *Pneumocystis carinii*, BAL fluid was centrifuged for 3 to 5 minutes at 1,200 rpm. The sediment was smeared and stained with Gram-Weigert stain and read under the microscope on high power.

RESULTS

Characteristics of Patient Population. The sample consisted of 21 children: 9 boys (43%) and 12 girls (57%). They ranged in age from 3 months to 17 years. All had diffuse pulmonary infiltrates or interstitial disease that failed to improve or worsened despite therapy. All (100%) were on broad-spectrum antimicrobials; 2 (10%) were on antiviral therapy at the time of BAL; most patients had completed 10 to 14 days of therapy prior to BAL. Ten of 21 (48%) had documented causes of immunosuppression. An additional 5 of 21 (24%) had anatomic abnormalities identified as causative or contributing factors leading to recurrent pneumonia requiring repeat hospitalizations. One of 21 (5%) had documented exposure to pulmonary tuberculosis. The remaining 5 of 21 (24%) had no documented immunologic disorder.

Isolates in Sputum. Fifteen of 21 (71%) children had sputum samples sent for microbial evaluation. The identification of one or more organisms was determined to be a "positive yield." No identification or identification of normal mouth flora only was labeled a "no yield." The overall yield for isolation of any pathogen in sputum was 13 of 15 (87%) children. However, there were a total of 69 tests performed, ie, cultures inoculated. Forty-two of 69 (61%) of cultures revealed no growth. Isolates were identified in 27 of 69 (39%; Table 1).

Isolates in BAL. Similarly, the isolates obtained from BAL can be examined. All 21 (100%) children underwent BAL. The identification of one or more organisms was determined to be a "positive yield." No identification or identification of normal mouth flora only was labeled a "no yield." The overall yield for isolation of any pathogen in BAL was 20 of 21 (95%) children. However, there were a total of 165 tests performed, ie, cultures inoculated. One hundred twenty-five of 165 (76%) cultures showed no growth. Isolates were identified in 40 of 165 (24%; Table 2).

Comparison of BAL to Sputum. In comparing BAL to sputum isolates, results were concordant in 6 of 15 (40%). In 9 of 15 (60%) there were discordant yields. Of the 60% in which discrepancy was noted, in 20% (n = 3) the sputum yield was greater, in 20% (n = 3) the BAL yield was greater, and in 20% (n = 3) a difference was noted; ie, additional information was obtained from BAL, but organisms isolated from sputum cultures were not confirmed. Therefore, in 9 of 15 (60%) children, noninvasive sputum sampling provided all the same data as the more invasive BAL technique.

DISCUSSION

Overall, for any given patient, identification of at least one pathogen occurred in 87% (n = 13 of 15) of sputum samples and 95% (n = 20 of 21) of BAL specimens. These data conform to the range of values noted in the literature of 71% to 95%.[4,6]

Of those patients who had sputum sent for culture (n = 15), 69 cultures were inoculated and only 27 (39%) demonstrated growth. Similarly, in those patients who underwent BAL (n = 21), 165 cultures were inoculated and only 40 (24%) revealed growth.

TABLE 2. BRONCHOALVEOLAR LAVAGE ISOLATES (N = 21)

	RSV	*Bact*	*PMN*	*PCP*	*AFB*	*CMV*	*Fungus*	*KOH*	*Pert*	*Leg*	*Cand*
Positive	4	10	10	0	1	4	1	1	0	0	9
Negative	13	11	8	15	18	10	11	12	16	11	0
N/A	4	0	3	6	2	7	9	8	5	10	11

Percent yield was 20 of 21 or 95%.

N/A — culture not obtained, RSV — respiratory syncytial virus, Bact — bacteria, PMN — polymorphonuclear cells, PCP — *Pneumocystis carinii*, AFB — acid-fast bacteria, CMV — cytomegalovirus, KOH — yeast, Pert — *Bordetella pertussis*, Leg — *Legionella*, Cand — *Candida albicans*.

This suggests that the majority of cultures inoculated show no growth. In 9 of 15 (60%) patients who had both sputum and BAL obtained for culture, sputum sampling provided the same information as BAL, or more.

In evaluating our results, one may note several findings. Cytomegalovirus was only isolated from BAL specimens. *Candida albicans* had a greater yield in our cultures from BAL than sputum. No *P carinii* or *Legionella* was identified from BAL or sputum samples. The remainder of our data demonstrate that various organisms were not remarkably better isolated from either sputum or BAL.

One possible variable affecting culture results is that of oral contamination in the samples. Generally, random sputum specimens are believed to have a higher incidence of oral contaminants than induced samples. In health, the lower respiratory tract is sterile. Contaminants may be introduced on intubation for mechanical ventilation and/or diagnostic intervention.

The differentiation of pathogen from contaminant is nearly impossible. One cannot rely on an elevated neutrophil count to diagnose acute infection, because counts may be elevated in such conditions as active pulmonary fibrosis or adult respiratory distress syndrome, and they may be depressed in immunocompromised patients.[10] Attempts to distinguish pathogens from contaminants may be aided by the use of quantitative bacteriologic studies (ie, samples with $\geq 10^5$ colonies and $<1\%$ squamous epithelial cells are less likely to be contaminated).[10,11] In addition, protected brushings can be used during BAL in adults, but are not often feasible in children, secondary to technical limitations in a significantly smaller airway.

There is an 83% isolation rate of fungus through BAL reported in the literature.[8] There is, however, a low rate of isolation of acid-fast bacilli with BAL in children, as was noted in our patient population. This low isolation rate utilizing BAL has been attributed to the observation that children with primary pulmonary tuberculosis have closed, caseous lesions with smaller numbers of mycobacteria in comparison to adults, and that the use of lidocaine in awake patients may reduce the yield for recovering mycobacteria.[12,13] Abadco and Steiner,[12] in a 1992 study, compared BAL to gastric lavage for the diagnosis of *Mycobacterium tuberculosis* in children. They found gastric lavage performed on three consecutive mornings to be superior to BAL in diagnosing acid-fast bacilli. Our results, as well as a review of the literature, would support the initial use of gastric lavage in those patients with suspected *M tuberculosis*.

A review of the literature confirms *P carinii* pneumonia to be the most common isolate from BAL in immunosuppressed hosts, regardless of the cause of the immunocompromise.[1,2,8,10,14-16] Yet, in this retrospective review, no *P carinii* was isolated. None of the patients with human immunodeficiency virus (n = 3) were found to be infected with *P carinii*. A possibility for the low yield in the isolation of *P carinii* in this study may be that BAL was selectively not performed in patients with *P carinii* pneumonia. *Pneumocystis carinii* is readily isolatable with routine suctioning; therefore, the diagnosis may have been made without the need for BAL.

Identification of acute CMV infection has been described as technically difficult, because the mean culture time is 27 days and a wide spectrum of genetic and structural variability makes monoclonal antibody detection challenging. Cytomegalovirus has the ability to create multifocal infection with uneven distribution among cells. Hence, the number of CMV isolates does not reliably predict the severity of disease or differentiate acute infection from colonization or shedding.[14,15]

CONCLUSION

The data in this study support the utility of BAL in the armamentarium of tools available for the diagnosis of diffuse pulmonary infiltrates. Yet, the operative and laboratory expenses of both sputum sampling and BAL seem excessive. In light of the present-day political agenda to limit medical expenditures, what is the role of BAL in hospitalized pediatric patients? It may be an acceptable and economically plausible alternative to begin broad-spectrum intravenous antimicrobials for pneumonia (without sample for culture), as we might for sinusitis or pharyngotonsillitis, and reserve diagnostic intervention for those who fail.

Based on this 1-year retrospective review at The New York Hospital–Cornell Medical Center, the following recommendations are suggested.

1. Broad-spectrum intravenous antimicrobials should be instituted in patients not responding to oral agents.

2. Noninvasive sputum samples should be obtained on all patients who do not improve or worsen with institution of broad-spectrum intravenous antimicrobials. In those patients who are mechanically ventilated, deep suction lavage may be obtained.

3. Bronchoalveolar lavage should be reserved for those patients in whom *Candida*, CMV, or *Bordetella pertussis* is suspected. Bronchoalveolar lavage is

also indicated for those patients in whom sputum cannot be obtained, or when the presence of structural abnormalities must be evaluated.

4. Gastric lavage should be utilized in children suspected of having tuberculosis.

5. Cultures ordered should be limited by such variables as immune status and the time of year in which the infection occurs.

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FOURTH ANNUAL GOLF SEMINAR — ADVANCES IN OTOLARYNGOLOGY–HEAD AND NECK SURGERY

The Fourth Annual Golf Seminar — Advances in Otolaryngology–Head and Neck Surgery will be held March 11-16, 1997, at the Pinehurst Resort & Country Club in North Carolina. For information, contact Leslie Sullivan, Coordinator, University of Washington School of Medicine Virginia Merrill Bloedel Hearing Research Center, Box 357923, Seattle, WA 98195-7923; E-mail sullivan@u.washington.edu; telephone (206) 685-2962; fax (206) 616-1828.

Ann Otol Rhinol Laryngol 105:1996

EFFECTS OF PERITONSILLAR INFILTRATION ON POST-TONSILLECTOMY PAIN

A DOUBLE-BLIND STUDY

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The concept that local infiltration of the operative area with a local anesthetic when using general anesthesia could alleviate postoperative pain is well known. We tested this concept on 129 patients scheduled for elective tonsillectomy. The patients were investigated in a double-blind, randomized study, and the operation was carried out via the standard technique of infiltrating the peritonsillar area preoperatively. The results indicated that preincisional infiltration of the tonsils with bupivacaine hydrochloride markedly decreased the intensity of pain following tonsillectomy, well beyond the immediate postoperative period.

KEY WORDS — bupivacaine hydrochloride, tonsillectomy.

INTRODUCTION

In the last few years, electrophysiologic studies have demonstrated that a noxious stimulus may activate afferent C fibers and induce long-lasting changes in the excitability of dorsal horn neurons.[1,2] These nociceptive impulses could contribute to postoperative pain.[3] The concept that local infiltration of the operative area with a local anesthetic during inhalational anesthesia could alleviate postoperative pain has been known since the beginning of the century.[4]

In 1990 Tverskoy et al[5] reported that postoperative pain after inguinal herniorrhaphy was profoundly decreased when surgery was performed with the addition of a local anesthetic. In 1991 Jebeles et al[6] reported that preincisional infiltration of the tonsils with bupivacaine hydrochloride markedly decreased the intensity of pain following tonsillectomy. Their study was based on 14 patients scheduled for tonsillectomy who were randomly divided into two groups. In one, the tonsillar fossae were infiltrated with normal saline with epinephrine, and in the other, the tonsillar fossae were infiltrated with bupivacaine.

The aim of the present study was to assess the role of a preincisional infiltration of tonsils with bupivacaine in decreasing post-tonsillectomy pain.

MATERIALS AND METHODS

One hundred forty-seven patients, 6 to 25 years of age, scheduled for elective tonsillectomy or tonsillectomy and adenoidectomy, entered the study. These patients were investigated in a double-blind, randomized study, and the operation was carried out by means of one standard technique.

All patients received 0.006 mg/kg atropine, 4 mg/kg pentobarbital sodium, and 0.5 mg/kg atracurium besylate for muscular relaxation. After oral intubation, anesthesia was maintained with fentanyl citrate, oxygen, and 67% nitrous oxide. The usual physiologic parameters (respiratory rate, pulse, blood pressure, and temperature) were monitored during surgery. Following the induction of general anesthesia, but prior to the onset of surgery, the peritonsillar regions were infiltrated.

The patients were randomly assigned to one of two groups (see Table). In one group (the bupivacaine group), the tissues surrounding the tonsils were infiltrated with 0.25% bupivacaine hydrochloride with epinephrine (1:200,000), 3 to 5 mL per tonsil. In the other group (the saline group), the tissues surrounding the tonsils were infiltrated with normal saline with epinephrine (1:200,000). No narcotic was used

PATIENT CHARACTERISTICS (N = 147)

	Saline	*Bupivacaine Hydrochloride*	*Total*
Males	35	36	71
Females	38	38	76
Age 5-14 years	49	47	96
Age 15-25 years	28	23	51

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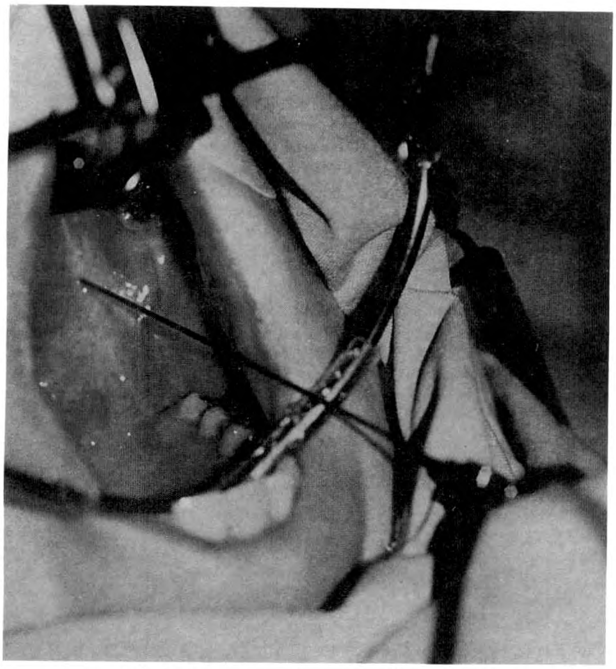


Fig 1. Injection of peritonsillar areas.

for premedication or after surgery.

All infiltrations were performed in the same manner. The needle was inserted very superficially into the tonsillar pillar, and following aspiration the pillar was ballooned (Fig 1). Three injections into both pillars were made on every patient: at the superior pole, at the inferior pole, and between the poles. These six injections displaced the tonsils toward the midline and helped to enhance the plane of dissection.

All tonsillectomies were performed by means of the dissection and snare technique.

The day before surgery the patients were instructed on how to use a visual analog scale (VAS) that consisted of a 100-mm horizontal line marked "no pain" at its left and "worst pain" at its right end.[7] The nurse helping each child with the assessments was blinded to the patient group. A modified version of the VAS for children was prepared, to simplify their answer (Fig 2). Two types of pain were assessed: constant pain (in the absence of any pharyngeal movement) and pain caused by drinking 100 mL of water. Each patient rated his or her pain, with the help of the nurse, every 4 hours for 24 to 48 hours following surgery. (Hospitalization time for tonsillectomies in our department is 48 hours.)

The VAS results were analyzed by the Mann-Whitney test. A p level of $< .05$ was considered significant.

RESULTS

The study groups were comparable with respect to age and sex (see Table). Eighteen patients (of 147) who inadvertently received analgesics after the operation and therefore did not conform with the strict postoperative rules of the study were excluded.

The results of the visual analog scores were divided into three main groups (Fig 3). The first group ($N = 16$) represented no pain or almost no pain post-tonsillectomy (VAS of 1 or 2), the second group ($N = 67$) represented medium pain (VAS of 3 or 4), and the third group ($N = 46$) represented severe pain (VAS of 5).

The first group (VAS of 1 or 2) included 2 patients who received normal saline and 14 who received bupivacaine. All these patients suffered almost no pain after the operation. The difference between saline- and bupivacaine-treated patients was statistically significant ($p < .05$).

The second group (VAS of 3 to 4) included 32 patients who were injected with saline and 35 with bupivacaine. All of them complained of medium pain, and some of them needed analgesics. This difference was not statistically significant.

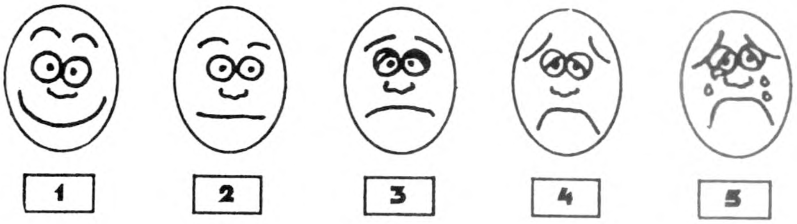
The third group (VAS of 5) included 18 patients injected with bupivacaine and 28 with saline. All of them suffered from severe postoperative pain and needed analgesics. This difference also was not statistically significant.

We did not notice any considerable difference between children and adults in the reduction of pain.

DISCUSSION

The addition of local anesthesia to general anesthesia by injection of the peritonsillar area resulted in decreased intensity of pain following tonsillectomy. Similar results were obtained with postoperative pain

Fig 2. Simplified version of visual analog scale.



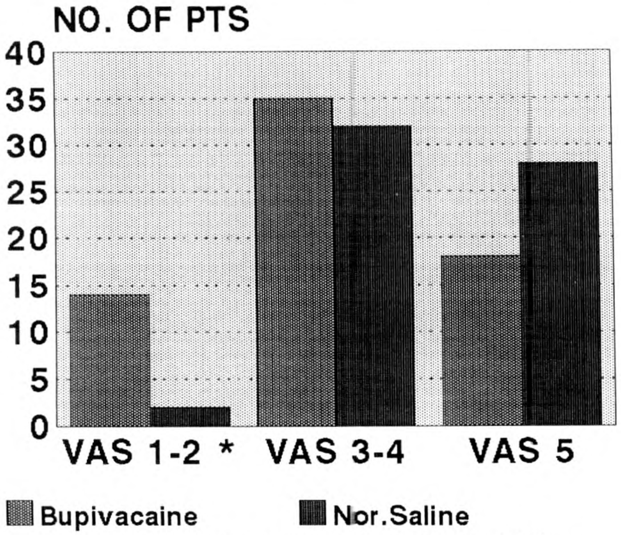


Fig 3. Results of postoperative pain complaints. VAS — visual analog scale (1 — least severe pain, 5 — most severe pain), * — $p < .05$.

after inguinal herniorrhaphy: the pain intensity was profoundly decreased when surgery was performed with the addition of local anesthesia.[5]

Many anesthesiologists are reluctant to allow surgeons to inject epinephrine-containing solutions during the administration of volatile general anesthetic because of the fear of precipitating cardiac ectopy.[8] None of the patients in our study developed ectopy.

The duration of infiltration anesthesia with 0.25% bupivacaine usually lasts several hours.[9] If a single (preincisional) bupivacaine infiltration of the tonsils resulted in decreased postoperative pain intensity for 5 days, the pain relief clearly cannot be explained by the prolonged presence of the local anesthetic in the area of surgery. An explanation for this long-lasting pain relief might be that neural blockade prevents nociceptive stimulus impulses from entering the central nervous system during and immediately following surgery and thus suppresses formation of the sustained hyperexcitable state responsible for the maintenance of postoperative pain.

Two groups of investigators have recently studied the effect of infiltration anesthesia on post-tonsillectomy pain.[8,10] Broadman et al[8] assessed the effect of bupivacaine immediately after surgery, when the direct pharmacologic effect of the local anesthetic should have been present; however, analgesia was reported only by Pappas et al.[10]

CONCLUSION

The results of our study indicate that preincisional infiltration of the tonsils with bupivacaine markedly decreases the intensity of pain following tonsillectomy, well beyond the immediate postoperative period.

ACKNOWLEDGMENTS — The authors thank Rachel Zur, RN, and Rosi Kassab, RN, for their assistance and follow-up in the double-blind study, and Ruth Singer for her secretarial work.

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Ann Otol Rhinol Laryngol 105:1996

ALLOGRAFT TYMPANOPLASTY TYPE 1 IN THE CHILDHOOD POPULATION

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Seventy allograft type 1 tympanoplasties in children under 16 years of age were studied retrospectively. Only patients with a tympanic membrane perforation without cholesteatoma and a normal ossicular chain were included. Short-term and long-term anatomic and functional results were analyzed after a mean follow-up of 40 months. The overall short-term take rate was 97% and the long-term take rate was 88%. A number of variables with alleged prognostic value for surgical outcome were analyzed, but none showed statistical correlation with either anatomic or functional results. A median hearing gain of 10 dB was achieved, which was stable over time. We conclude that allograft tympanoplasty type 1 in this group of children is anatomically and functionally successful regardless of age.

KEY WORDS — allograft tympanoplasty, child, chronic otitis media, homograft tympanoplasty.

INTRODUCTION

A surgical treatment of chronic or recurrent otitis media without cholesteatoma, type 1 tympanoplasty[1] in children has always been controversial. The controversy concerning the timing of surgery is mainly based on valid arguments from both the literature and the experience of many surgeons. Immature aeration of the middle ear, recurrent upper respiratory tract infections, and a difficult surgical anatomy have a negative influence on surgical outcome.[2,3] On the other hand, early restoration of auditory function and prevention of further progression of middle ear disease (and possibly progression to cholesteatoma) caused by recurrent otorrhea are strong arguments in favor of early tympanoplasty.[4,5] This controversy is certainly enhanced by the variable success rates mentioned in the literature, due to large differences in selection criteria, definition of success, and length of follow-up.

Allograft (or homograft) tympanoplasty was first introduced by Jean Marquet[6] in 1966. He advocated the use of allograft tympanic membrane and ossicles in order to obtain an optimal anatomic and functional reconstruction of the middle ear in chronic middle ear disease (Fig 1). The present authors were trained by him, and as did J. Marquet, they also routinely transplant tympanic membranes in children.

This paper reports on a retrospective analysis of the anatomic and functional results of type 1 tympanoplasty in the childhood population. It does not address the alleged risk of transfer of infectious diseases, namely, Creutzfeldt-Jakob disease (CJD) and human immunodeficiency virus (HIV). Transmission of CJD has never been reported after transplantation of other than brain tissue or cadaveric dura mater. It is reported in 10 cases after the implantation of dura mater grafts.[7] In addition, the incidence of CJD is extremely low (1 per 1 million), and stringent criteria for donor selection should exclude donors who are at risk for CJD. No cases of transmission of HIV have been reported with nonvital allograft material. Preservation with formaldehyde is accepted to eliminate HIV.[8]

MATERIALS AND METHODS

For the present retrospective study, the consecutive files (November 1982 to November 1993) of children who underwent tympanoplasty type 1 under the age of 16 were retrieved and numbered 78. Only patients with a tympanic membrane perforation with-

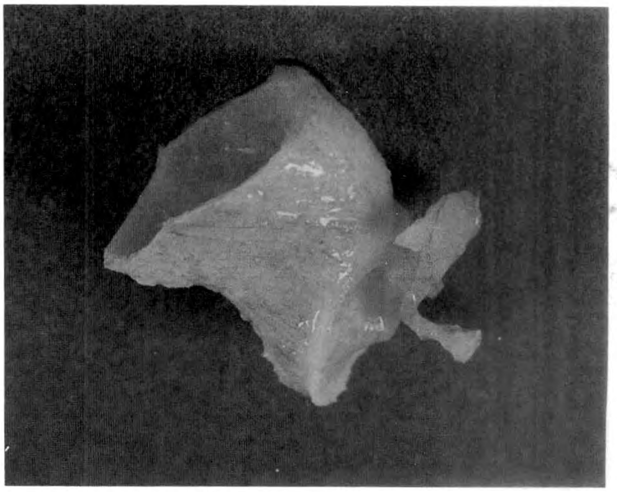

Fig 1 . Tympano-ossicular allograft.

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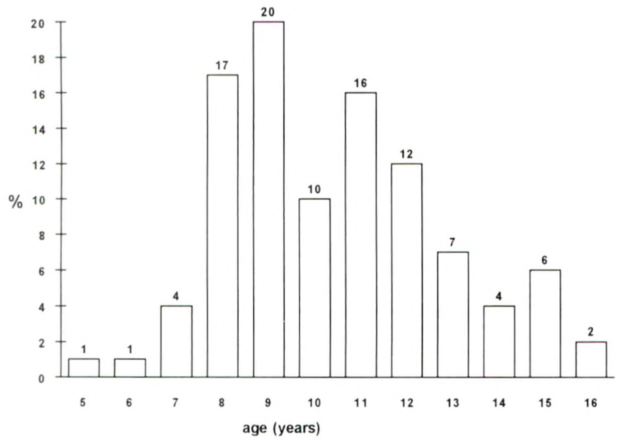

Fig 2. Age distribution at time of surgery.

out cholesteatoma and with a normal ossicular chain were selected. Revision surgery was excluded, as well as retraction pockets and cases operated on with the underlay technique. Of these 78 operations, 8 were excluded because they were considered statistically dependent cases because the other ear had already been enrolled in the present study. In such a case, a random choice was made as to which ear to include. Hence, 70 ears remained for the present statistical study.

Surgery was performed under general anesthesia with hypotension. The technique of allograft tympanoplasty according to Marquet[9] was used. Briefly, a retroauricular incision and dissection of the skin and of the external auditory canal is performed, followed by a total myringectomy. An allograft tympanic membrane is fitted in the bony annulus, and the skin of the external auditory canal is repositioned over the cuff of the allograft. An external ear canal packing with an antibiotic ointment is left in place for 1 week.

Allograft tympanic membranes were harvested and prepared in the temporal bone bank of St Augustinus Hospital, according to the standards prescribed by Belgian law (Belgisch Staatsblad, 13/6/1986). The grafts were preserved by being left in formaldehyde for at least 2 weeks followed by Cialit (Hoechst Pharmaceuticals) for a minimum of 3 weeks.

Nineteen variables were studied, namely, age, sex, side, surgeon, cause and site of the perforation, state of the mucosa, state of the contralateral ear, and history of mastoidectomy and adenoidectomy, together with three postoperative evaluations (at 3 and 12 months and the latest evaluation available) in terms of state of the graft, presence of perforation, and state of the outer ear canal. Whenever available, four full audiometries (bone and air conduction) were analyzed: the preoperative audiogram, the audiograms at 3 and 12 months postoperatively, and the latest audiogram. Audiometry was performed in a soundproof room with an audiometer calibrated according to ISO (International Standards Organization) standards. A lack of response to air-conducted sound was coded as 120 dB, and to bone-conducted sound as 80 dB. Missing values were coded as such. Descriptive statistics were performed on all variables. Counts, percentages, and histograms were used to describe nominal data.

TABLE 1. SITE AND SIZE OF PERFORATION

Site and Size of Perforation	*% of Cases*
Small anterior	6
Small posterior	15
Large anterior	10
Large posterior	24
Large inferior	14
Subtotal	31

Small — restricted to one quadrant, large — restricted to two neighboring quadrants, subtotal — occupying more than two quadrants.

Hence, audiometric results (often expressed as the Fletcher index, ie, the mean of hearing levels at 500, 1,000, and 2,000 Hz) were described by means of nonparametric statistics. Analytic statistics were performed to study the interdependence of all variables and the dependence of the audiometric results on the different variables. The null hypothesis of independence was tested for each two separate variables. We used t-tests for independent variables, χ^2 tests, and ANOVA tests (analysis of variance). All tests were carried out two-tailed.

A Kruskal-Wallis analysis of variance was used to test the dependence of the audiometric results on the different variables. A Pearson correlation analysis was used to test the dependence of the audiometric results on age. All tests were carried out two-tailed. A global level of significance for the whole present study of 5% was set forward. In consequence, according to the Bonferroni principle, a much more stringent significance level was set for each individual test and only p values of 0.1% were considered significant. Taking into account an alpha error of 0.001 and a beta error of 0.20, the present study design was able to demonstrate audiometric differences of approximately 10 dB. All statistics were performed by a computer running CSS/pc software (release 2.1; Statsoft Inc).

RESULTS

The male-to-female ratio was 59:41. The right-left distribution was 53:47. The mean age was 10.3 years (range 5 to 16 years). The mean follow-up was 40 months (range 1.6 to 132 months). The age distribution at the time of surgery is shown in Fig 2. The site and size of the perforation are shown in Table 1. The

TABLE 2. ANATOMIC RESULTS IN TERMS OF STATE OF GRAFT DURING FOLLOW-UP

	% of Cases		
State of Graft	*3 Months*	*12 Months*	*Latest Evaluation*
Normal graft	88	82	74.5
Retraction	1.5	1.5	1.5
Lateralization without FRs	4.5	7.5	3
Lateralization with FRs			6
Edema of graft	0	0	1.5
Granulation on graft	3	3	1.5
Perforation	3	6	12

Lateralization — (partial) loss of contact between malleus handle and graft, FRs — functional repercussions, edema — thickening of graft with incomplete epithelialization.

perforation was a sequela of chronic otitis media in 87%, of the presence of a ventilation tube in 10%, and of trauma in 3%. The state of the contralateral ear was normal in 52% of cases. In 14% the contralateral ear showed active aeration problems such as retraction, atelectasis, or effusion. In 23% a contralateral perforation was present, and in 11% the contralateral ear was normal after previous surgery that had been performed for chronic otitis media. None of the contralateral ears showed cholesteatoma. The state of the middle ear mucosa at the time of surgery was normal in 86%, discharging in 2%, and edematous in 12%. In 10% of cases surgery was combined with mastoidectomy. Information about adenoidectomy was available in only 31 cases, in the majority of which (94%) adenoidectomy had been performed earlier.

The anatomic results in terms of state of the graft and reperforation of the graft are presented in Table 2 and Fig 3. The outer ear canal showed stenosis at 3 months in 1 case, which was treated with good results, and edema in another case. The later evaluations showed no outer ear canal abnormalities.

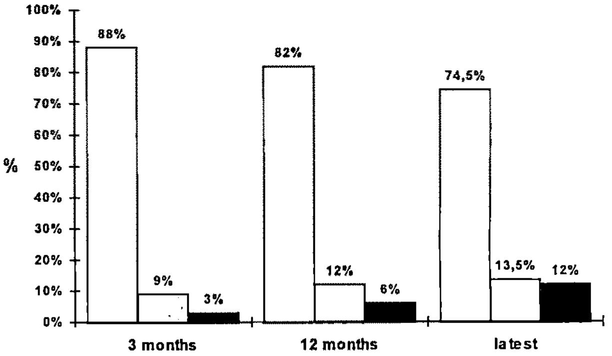

Fig 3. Anatomic results in terms of state of graft. White — normal, shaded — intact with complication, black — perforation. (Complications are specified in Table 2.)

The audiometric results at 3 months and 12 months are presented in Fig 4. The median preoperative bone and air conduction thresholds and the postoperative air conduction threshold are presented over all frequencies. The median hearing gain is 10 dB at 3 and 12 months and at the latest evaluation.

No significant correlation between the different variables was found, except for the contralateral ear, which in older children presented more often a history of previous surgery for chronic otitis media than in younger children (p = .005).

DISCUSSION

The success rate of type 1 tympanoplasty in children varies among the different authors. This is due to differences in selection criteria, definition of success, and length of follow-up. There is no study in the literature using exactly the same inclusion criteria for evaluation of success of type 1 tympanoplasty in children. All authors on this subject have studied a heterogeneous group including dry perforations, ossicular chain damage, revision surgery, and bilaterally operated cases.

When defining anatomic success of type 1 tym-

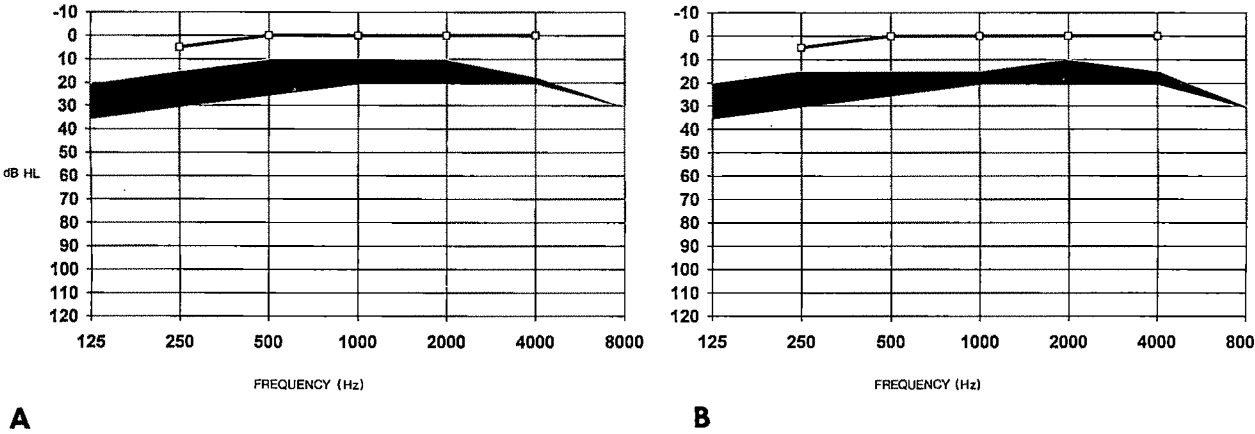

Fig 4. Audiometric data. Preoperative bone conduction thresholds (black line) and preoperative and postoperative air conduction thresholds (lower and upper borders of black area). A) After 3 months. B) After 12 months.

panoplasty as a nonperforated graft, our short-term success rate is 97% and long-term success rate 88%. The success rates of other papers vary from 35%[2] to 93%[10] because of different definitions of success. Bluestone et al[2] considered tympanoplasty to have failed when negative middle ear pressure or serous effusion returned postoperatively. Tos[11] used the audiologic outcome to report surgical success in a population with heterogeneous disorders. It is intuitively logical that surgical outcome will vary with the severity of disease. Papers that can be well compared with the present study in terms of inclusion criteria and study design report success rates of 66% (follow-up 25.2 months),[12] 73% (follow-up 23.4 months),[13] 78% (follow-up 72 months),[14] and 81% (follow-up 19 months).[15] In all these papers, temporalis fascia was used as graft material in underlay. Compared with these papers, we report the highest success rate, with a long-term mean follow-up of 40 months. It is remarkable that papers including patients with more advanced middle ear disease, such as ossicular chain damage, report comparable success rates.[16-19]

A possible cause of failure after tympanoplasty is the development of secretory otitis media as a result of insufficient aeration of the middle ear. There is no reliable method for preoperative evaluation of the middle ear aeration, and therefore, the risk of postoperative secretory otitis media is hardly predictable. The status of the contralateral ear may be an indication,[12,20] although several authors reported that this factor had no predictive value for success of tympanoplasty.[15,18,21,22] In the present study no correlation was found between the status of the contralateral ear and surgical outcome, but the relatively small sample size of the subgroups renders statistical analysis inconclusive.

In the present study only 1 case presented with a perforation 4 years postoperatively after an episode of acute otitis media with purulent otorrhea. Four cases presented with a perforation after an episode of granulation on the graft, treated with local antibiotics. In 2 of these cases, granulation appeared 3 years postoperatively. In 1 case the perforation was preceded by stenosis of the outer ear canal, which was treated with good results.

Information on antibiotic administration preoperatively and postoperatively was not sufficiently available to include it as a variable in this study. Antibiotics were not given systematically. A possible effect of antibiotics on the surgical outcome has never been proven.

Previous studies have noted late graft failures even after many years.[14,21,23,24] The pathogenesis of these late failures can be related to middle ear aeration deficiency, which was in many cases the original cause of the perforation. An episode of acute otitis media can result in perforation of the graft, even after a long time. Clearance deficiency of the external ear followed by granulation tissue, with subsequent perforation, unrelated to the aeration capacity of the middle ear, could be considered another possible cause of late postoperative perforations. Therefore, strict short-term follow-up for local control of the graft and outer ear canal during the first 6 months is very important. It is our policy to forbid swimming for 6 months and flying for 2 months. Annual control until the age of 16 is recommended.

Besides perforation, an allograft tympanic membrane can show other anatomic problems (Table 2). A retracted graft (no retraction pocket) was found in 1.5% on both short-term and long-term evaluation. MacDonald et al[16] report 5 of 29 cases to show postoperatively retracted grafts after fascia myringoplasty.

An alleged complication of this surgical technique is lateral displacement of the graft. We define lateral displacement as the otoscopic situation of partial or complete loss of contact between the tympanic membrane and the malleus handle. This situation was found in 4.5% of cases at the short-term evaluation. It is evident that anatomic and functional results cannot be considered separately. Therefore, at the long-term evaluation a distinction was made between lateral displacement with and without functional repercussion. As presented in Table 2, 4 of 6 cases (6%) showed lateral displacement with functional repercussion (hearing unchanged or diminished). Of these 4 cases, only 1 showed a complete loss of contact between the graft and malleus, and 3 cases a partial loss of contact with the malleus handle. Two cases (3%) developed partial lateral displacement with normal hearing improvement as seen in the cases with a normal graft. These 2 cases had neither a functional nor a hygienic problem and the displacement cannot be called a complication.

In the literature, controversy exists about the timing of tympanoplasty in children with a dry perforation. The present study shows that tympanoplasty was performed from the age of 5 years. No correlation was found between age and surgical outcome. Of course, there was a tendency to delay surgery in cases of recurrent upper respiratory tract infections, adenoid hypertrophy, or nasal allergy, which were treated first. This tendency is nicely expressed by the 86% dry ears at the time of surgery. Still, no correlation was found between status of the middle ear mucosa and success rate. Some authors do not recommend tympanoplasty under the age of 10 years.[2,12,16,25] Shih et al[26] and Berger et al[21] reported more failures under the age of 10 years. Sheehy and Anderson[27] do not recommend elective surgery before 7 years and Raine and Singh[23] suggest waiting to 12 years. Several authors state that age is of no significant influence.[15,17-20,22,28] Smyth and Hassard[4] recommend myringoplasty as early as possible. According to our results there is no need to delay surgery until a "magic age." If a child presents with favorable conditions as mentioned before, surgery can be performed as soon as possible. The cause of the perforation showed no correlation with graft take. There was a tendency for the large perforations to be less successful, but the correlation was not significant. Possibly, the size of the perforation is a measure of the stage of middle ear disease. More advanced middle ear disease could have a higher failure rate after tympanoplasty. In contrast with the fascia temporalis underlay technique, the site and size of the perforation are of no importance in the technique of allograft type I tympanoplasty, which is identical in all cases. Hearing function is hardly ever the main indication for allograft tympanoplasty type 1 in dry perforations, yet it is a factor to be evaluated.

None of the ears presented with a postoperative sensorineural hearing loss or facial nerve palsy. The median preoperative air conduction threshold (Fletcher index) was 22 dB. No statistical significance was found between the named variables and the audiologic outcome. The median hearing gain at 3 and 12 months and at the latest evaluation was 10 dB. Of the four comparable papers mentioned earlier, only two report useful audiometric data.[14,15] Kessler et al[15] report that 90% of cases have a postoperative air-bone gap of 20 dB or less. No preoperative data were mentioned. Blanshard et al[14] report a closure of the air-bone gap to within 10 dB, averaged over the frequency range 250 Hz to 4 kHz, in 18% at the long-term follow-up (6 years). Other papers are hardly comparable on their audiometric results, because they include ossicular chain damage and revision surgery. These factors have too much impact on audiometric data for comparison with the population of the present study.

In conclusion, we state that allograft type 1 tympanoplasty is anatomically and functionally successful in this selected group of children, regardless of age. The functional success of allograft tympanoplasty is at least comparable to that of fascia tympanoplasty (according to literature reports), whereas the take rate appears to be higher. There is no obvious influence of the different variables postulated to affect the surgical outcome of type 1 tympanoplasty. This might be due to the small sample size of the subgroups.

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THIRTY-SECOND POSTGRADUATE COURSE IN EAR SURGERY

The Thirty-Second Postgraduate Course in Ear Surgery will be held in Nijmegen, the Netherlands, from March 31 to April 5, 1997. For information, contact Prof P. van den Broek, University Hospital Nijmegen, Department of Otorhinolaryngology, PO Box 9101, 6500 HB Nijmegen, the Netherlands; fax 024-3540251.

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TWENTY-FOURTH NES CONGRESS

The Twenty-Fourth Ordinary Congress of the International Neurootological and Equilibriometric Society Reg. (NES) will be held April 6-10, 1997, in Haifa, Israel. For information, contact NES Congress President Prof Dr Ludwig Podoshin, Otolaryngology, Head and Neck Surgery, Bnai Zion Medical Center, PO Box 4940, Haifa 31048, Israel; telephone 972-4-359544; fax 972-4-371393.

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EARLY SOUND DEPRIVATION AND LONG-TERM HEARING

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The long-term effects of hearing loss in early life were analyzed by tests of central auditory function. A majority of individuals failed the Compressed Speech identification with statistically significant results. There was an impact on a minority of individuals evaluated by Dichotic Sentences; little impairment was noted through Speech Reception in Noise. Delayed maturation of the central auditory complex may improve these findings, although during the period of investigation a negative impact was measured. Other issues of diagnosis, remediation, and the consequences of short- and long-term deafness are discussed.

KEY WORDS — central auditory function, child, deafness, sound deprivation.

INTRODUCTION

The long-term effects of sound deprivation in early life have been established by many researchers.[1] These include the issues of auditory reception, language development, behavioral disorders, intellectual growth, and academic achievement.[1] We shall limit this investigation to the effects of prolonged conductive hearing loss on central auditory function.[2]

The exact degree of the negative impact due to hearing impairment on specific functions such as IQ scores cannot be measured with predictable accuracy. The variable impact on individuals, assuming equal talent, comparable innate skills, and identical sensory stimulation, would imply that the ultimate result is a summation of numerous and disparate factors. At the same time, many positive compensatory maneuvers may coexist to counteract the negative aspects of hearing loss.

The goal of this investigation is identification of the impact on complex hearing function later in life, well beyond the period of sensory deprivation. At that time the peripheral sensitivity that is evaluated by pure tones is normal. The relatively unsophisticated response to simple words is intact, and the general auditory function appears to be within normal range. This study evaluates higher intellectual functions for integration of dichotic information, central suppression of unwanted acoustic input against wanted verbal communication, and the recognition of time-altered common words.

We submit that verbal communication in societal interchange is influenced by the composite of an ideal auditory environment and is degraded by the negative impact of ambient noise. Additionally, an ideal rate of approximately 200 words per minute is more comfortably understood than speech at a greater density per unit time. Last, the speech quality may be degraded by poor articulation and inadequate intensity. Collectively, these attributes increase the difficulty to facilitate verbal auditory reception.

We suggest that the individual ability to function in these complex situations may be compounded by the partial loss of central maturation and bitemporal integration of the cortical auditory system during the early developmental stage of sensory input.

A summation of the many negative influences[1] that are unequally distributed among individuals results in a variable impact on auditory maturation. As a consequence, the ultimate auditory skills are not homogeneous; a wide range of function with significant deviation from the mean may occur in a segment of the total cohort. The subsequent data defend this concept; the fact that many are not impaired does not refute the hypothesis, but rather, reflects the many components essential to sensory maturation.

We further propose that performance levels of central auditory dysfunction will not be uniform. The duration and degree of hearing loss (negative influence) is counteracted by the positive factors of remediation, which include increased speech level, personal attention, sensory stimulation, natural inherent talent, and socioeconomic status. The equation contains so many unmeasurable components that the outcome must be unique.

Incidence and Duration of OME. The duration of otitis media with effusion (OME) varies according to author.[1] There is some bias due to the source of the data; for example, school screening studies probably differ from the hospital or clinic reference, where the severity of the loss requires medical or surgical correction. The data define the prevalence of persistent otitis media in children between 9.5% and 32% beyond 12 months.[1] Shorter-term prevalence with some

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resolution is noted at 4 weeks (20%), 6 weeks (15%), and 6 weeks (10%).[3] The data from intermediate duration, that is, 6 to 12 months, identify 15% with persistent disease.[4-6]

Age and OME. Epidemiologic studies disclose a prevalence of OME of about 13% from ages 1 to 6 years, which then declines to about 7% at 7 years[1]; this figure is assumed to drop further beyond this age.[1]

Hearing Loss and OME. The wide range of hearing loss due to OME extends from 0 to 60 dB hearing threshold level (HTL). Reported data from different sources reveal an average hearing loss between 15 and 20 dB HTL,[7,8] 27.6 dB,[9] and 27 dB HTL[10]—remarkably consistent results. By contrast, negative pressure identified by tympanometry (C pattern) without middle ear effusion may be associated with a loss of 10 dB HTL or greater.[9] A comparison of these data with the scale of hearing loss proposed by Northern and Downs[11] classifies these subjects with a mild hearing loss. Accordingly, we suggest that an input deficit exists at this level and may be compounded by the negative impact of conflicting environmental noise and the intensity of conversational speech. Although the Dunedin report[1] relied heavily on tympanometry, we observe that this technique does not measure hearing thresholds. Furthermore, the diagnoses of mixed conductive and sensorineural and pure nerve deafness are not identified by this method — a final failing of this clinically limited technique.

Specific deficits in auditory processing and electrophysiologic testing have been identified by many authors.[12] The report by Welsh et al[2] in 1983 described impairment shown by dichotic testing in 23% of individuals with previous long-term conductive hearing loss; 57% failed frequency-distorted speech beyond 2 SD, and difficulty with binaural fusion was found in 77% of the cohort. A higher signal-to-noise ratio was required for satisfactory verbal communication than in normal individuals, while selective auditory attention appeared to be deleteriously impaired. As a consequence, learning disorders were related to verbal information impairment.[1] Pillsbury et al[13] concluded that MLDs (masking level differences) were abnormally small and proposed that an inability to extract signals from noise with poor auditory processing resulted from chronic early ear disease. Significant prolongation of auditory brain stem response III and IV intervals was observed by Folsom et al[14] in 1983.

METHODS AND MATERIALS

Forty-five subjects, 6 to 21 years of age, were surveyed; the hearing threshold level was 10 dB or better, and speech discrimination (PB 50; phonetically balanced 50) exceeded 90%. In addition to basic audiometry, the authors utilized the dichotic competing sentence test of Willeford and Burleigh,[15] the test by Beasley et al[16] of monaural compressed speech, and the authors' test of discrimination in noise (40 dB sensation level and 5 dB signal to noise ratio).

The age distribution of the children was as follows: age, 6, 8 subjects; age 7, 7 subjects; age 8, 7 subjects; age 9, 4 subjects; age 10, 6 subjects; and age 11 and older, 13 subjects. All subjects had a reported history of middle ear effusion and hearing loss that occurred during the first 4 years of life and persisted for at least 1 year. All subjects had previously required middle ear tubes to resolve their otitis media, but they had normal hearing at the time of this testing by audiometric evaluation. Various descriptions of minor speech-language, reading, and other academic difficulties were given by parents and teachers alike. These were all described as normally intelligent children with no significant academic deficiencies.

RESULTS

Competing Sentences. The test identifies simultaneous binaural reception of different stimuli; the locus of dysfunction is isolated to the posterior region of the temporal lobe.[15] There are 37 subjects in this section; the norms were previously derived from 50 unimpaired subjects. Dominant and weak ears are described rather than right and left, since the sinistral is occasionally the strong ear. Normal maturation reveals a slow catch-up of the weak ear to the strong by age 11; the range of normal function is wide in the early years, particularly on the nondominant side.

The data are reported on the basis of standard deviation from the mean norms. Thirteen of the 37 individuals exceed 2 SD by 10% or greater. Reversal of dominance appears in a scatter fashion in each age segment, although the strong ear measures within the normal range in these subjects. Although the data are limited, a minority of subjects reveal a delay in maturation, but a trend toward gradual improvement is suggested (Fig 1).

Discrimination of Speech in Noise. Normal ears can readily extract meaningful speech from background noise; this function is considered to be a central auditory phenomenon. This simple test may reveal children with an auditory learning disability, and reflects an inability to hear comfortably in the presence of ambient speech.[17,18]

Forty-seven subjects were evaluated and compared to the normal mean scores of approximately

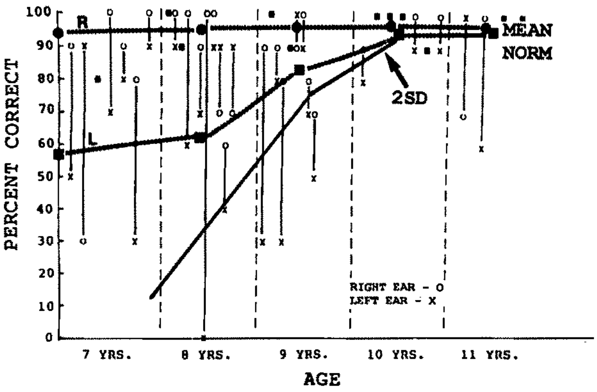

Fig 1. Normative data for right and left ears using Willeford's competing sentences are reported by age and demonstrate that while right ear is essentially stable over time, left ear improves with maturation. Solid line represents 2 SD from norms; individual scores are recorded from right and left ears.

94%. The standard normal deviations for ages under 8, 8 to 10, and 11 and older diminish from 5.9% to 2.8% with increasing age. The range of individual performance extends from 84% to 100%. Four of the 47 subjects scored in excess of 2 SD (less than 10% of the total). Other data (unpublished material by Welsh et al) indicate that as the signal-noise ratio increases beyond the 5 dB utilized in this study, a further separation in the data becomes apparent (Fig 2).

Word Compression and Intelligibility. Accelerated speech at 60% is usually recognized without difficulty in normally functioning ears; an impairment was reported in the learning-disabled, although the locus of dysfunction has not been defined.[16] The Beasley et al[16] test utilized CNC (consonant-nucleus-consonant) monosyllabic words; the spectral components are intact without frequency distortion; only the duration of exposure is modified. The normal mean values extend from approximately 70% (under 8 years) to 91% (beyond 10 years). The standard deviations are approximately 8% in the youngest and 6% in the eldest studied. Comparison to the test group reveals a wide range of response in each segment. Overall, 69% of the group fell within the 2 SD range; the remaining 14 individuals deviated widely from the mean values. This observation was most noticeable in the 8- and 10-year brackets (Fig 3).

DISCUSSION

An overview of the data from this investigation indicates that a negative performance scale greater than 2 SD was noted in 30% of the competing sentence test, 10% of speech in noise, and 41% of compressed speech. Comparison with the normal data revealed a wide disparity of function, particularly with respect to the maturation patterns of normal subjects. As a group, the data from accelerated speech testing were statistically significant, supporting a negative impact on the ability to understand rapid conversation. By contrast, the data derived from competing sentences and environmental noise were not, as a group, statistically significant; nonetheless, within this cohort, a segment illustrated impairment. We conclude that the long-term impact of verbal recognition with the masking effect of simultaneous noise seems to be negligible. The results from binaural identification of dichotic sentences continues to reveal poor maturation and a persisting weak ear.

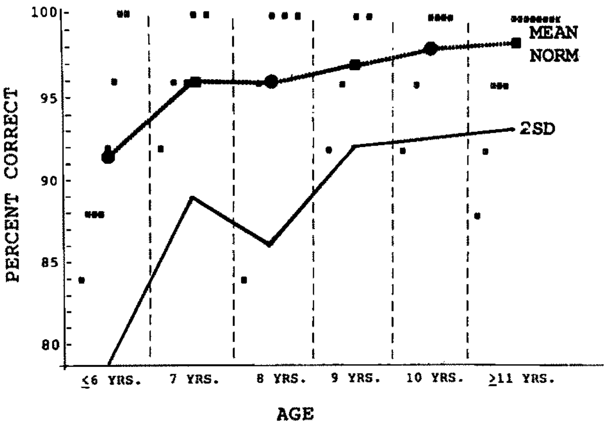

Fig 2. Discrimination in noise is reported from response to taped monosyllabic W-22 word lists presented at plus 5 dB signal-to-noise ratio embedded in speech noise. Normative mean values extend from 92% to 97%, demonstrating mild improvement with maturation. That few individuals fall below 2 SD suggests little impact on speech reception due to environmental noise.

As clinicians, we suggest that the prolonged hearing loss due to otitis media must be considered in the short and long term. The immediate impairment resulting from prolonged effusion is sound and verbal deprivation secondary to a loss of auditory sensitivity. The consequence is diminished speech input

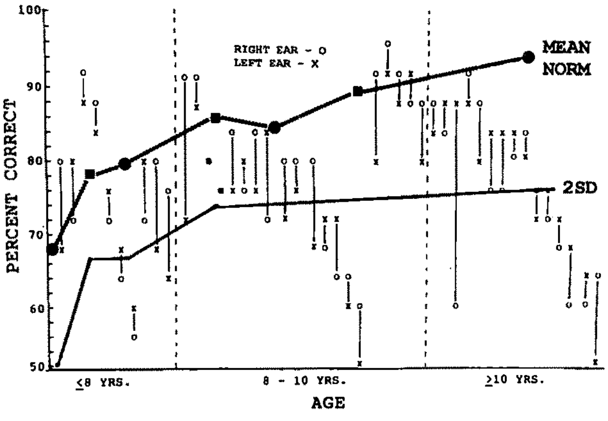

Fig 3. Beasley's compressed speech, set at 60% compression, was utilized in this study. Individual performance data are plotted with reference to normal mean values. That significant number of subjects fall below 2 SD suggests receptive dysfunction in hearing.

and an isolation from social and educational experience. The immediate damage may be reflected in delay in early vocabulary development, delay in speech, and limitation of intellectual potential. In the later period during the early school years, the loss of academic achievement, specifically in reading-oriented skills, has long been described.[19-33] Over the entire period, the problem of central reception of verbal data by the auditory system is recognized. Binaural integration of dichotic or diotic data, communication in competitive noise, identification of distorted speech, and extraction of verbal data from noise are abnormal in a minority of individuals.

The authors suggest that the performance data must be considered individually rather than by group or mean values. Since only a few fall outside the normal values, examination of the mean or group data would fail to identify these individuals. Of interest, these data and others[2] indicate that the patterns of central auditory disability identified by a battery of studies[15] reveal variable dysfunctions rather than a uniform characteristic of disability. We suggest that this phenomenon represents multifocal areas of functional involvement within the central auditory system. As part of the mechanism, we suggested a deleterious effect on high-frequency integration from dichotic verbal stimulation.[2] Further, we commented on the impairment of cortical integration, particularly in syllabic fusion studies — a second disturbance in the central auditory system.

Although this current study discusses the data of early hearing loss and the sequelae thereof and the many-faceted disabilities of cognitive and communication skills, we should certainly not overlook the impact of prolonged conductive hearing loss, whether of recent onset in grades kindergarten through fifth, or as an ongoing problem from infancy. While anecdotal, difficult to measure, and variable in degree, countless occurrences of teacher's concerns, parental anxiety, and relative failure in school confirm the immediate result of middle ear effusion and hearing loss.

We mentioned in our earlier report[2] that a predictable level of individual central auditory damage could not be determined from an analysis of these previous and current group data. The impacts of deprivation vary individually by the degree and duration of hearing loss and, finally, the positive maneuvers for compensation, ie, lipreading and speech education. Other authors[34,35] have subsequently echoed this attitude; in fact, we doubt that the problem of a quantitative assessment of dysfunction will ever be resolved. Factors of longitudinal study, sufficient numbers of subjects for statistical analysis, socioeconomic status, and the variability of research protocols are mentioned. The complexity of these issues and the quest to overcome these factors plagues our colleagues. Finally, the financial expenditure in personnel, subjects, and equipment may preclude a final resolution of the issue. In spite of these negative aspects, we suggest that preventive remediation be utilized against prolonged hearing loss. In conjunction with others,[36] we comment that the issues are of such importance that an aggressive approach must be taken to minimize the negative impact of sensory deprivation, specifically hearing loss.

ACKNOWLEDGMENTS — The authors thank Joseph Dragonette, PhD, Professor of Statistics, St Joseph University, for the data analysis, Walter Webb for the illustrations, and Marilyn Mongan for the typing and editorial comment.

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ADDENDUM

The central limit theorem was applied to the data from Competing Sentences and Speech in Noise for statistical analysis. This theorem states that a random variable that is the summation of several independent factors will have an approximately normal distribution of values. The chance of an excess of 2 SD above or below the mean is at most approximately .05, and for 3 SD, .0003. We conclude that an extreme individual measurement reflects some persistent or nonrandom cause, such as the multiplicity of factors involved in the development of and correction for sound deprivation.

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PREVALENCE OF SEVERE TO PROFOUND SENSORINEURAL HEARING LOSS IN CHILDREN HAVING FAMILY MEMBERS WITH HEARING IMPAIRMENT

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The purpose of this paper is to study the prevalence rate of hearing impairment among children with siblings and family members having hearing and speech disorders. A random survey of 6,421 Saudi infants and children between 2 months and 12 years of age was conducted in the Riyadh area. Children with no or few siblings and those who were firstborn were at relatively higher risk, and there was a strong association between children in the hearing-impaired group among those having living siblings with deafness. Those children who had family members with hearing and speech problems were at twice the risk of their counterpart groups regarding hearing impairment.

KEY WORDS — consanguinity, deafness, hearing loss, heredity.

INTRODUCTION

Research data on siblings, cousins, or family members with hearing disorders has led to an increased understanding of genetic causes of hearing impairment. Furthermore, the older a population of children, the better the information regarding younger siblings or cousins who might have been born with genetic hearing disorders. Childhood hearing impairment may be acquired or genetic (hereditary). The identification of hereditary deafness is reached by careful taking of family, pregnancy, birth, and infancy history, by careful physical examination of the affected individual and other family members, and by the necessary laboratory work. Hereditary hearing impairment may be conductive or sensorineural and may be congenital or delayed. Congenital forms appear at the time of birth, and delayed (heredodegenerative) hearing loss develops subsequent to birth. In this study, only severe (61 to 80 dB) to profound (81 dB or more in the better ear) sensorineural hearing loss is considered, with no attempt to differentiate between the congenital and delayed types. Hearing loss or impairment is defined as a loss of more than 25 dB in the better ear. The factors that identify those infants who are at risk for hearing impairment include the following.

1. Family history of childhood hearing impairment.
2. Prenatal and perinatal infections (ie, rubella, cytomegalovirus, syphilis, herpes simplex).
3. Anatomic malformation of the ear, head, and neck.
4. Birth weight less than 2,500 g.
5. Hyperbilirubinemia at a level exceeding the indication for exchange transfusion, depending on age and weight (340 µg/L or 20 mg/100 mL).
6. Bacterial meningitis.
7. Severe neonatal asphyxia.

In Saudi Arabia, few studies have been done recently. In those available,[1-3] etiology was not studied in detail. This paper will deal with the family history of hearing loss and will assess the correlations of hearing and speech disorders among siblings and family members and the prevalence of hearing impairment.

MATERIALS AND METHODS

A random sample survey of 6,421 Saudi infants and children below the age of 12 years was carried out in Riyadh, Saudi Arabia, for the 27-month period from May 1988 to September 1990. The sampling was designed to have an adequate and representative covering of all socioeconomic and demographic groups of the Saudi population living in Riyadh.

The sampling design was essentially a three-stage stratified random sampling using age and sex as stratifying factors in the final stage. The city was divided into 93 administrative areas and these areas were distributed into six strata according to socioeconomic homogeneity. One fifth of the areas in each stratum were chosen by a simple random method. Each area was further subdivided into roads and the latter were subsequently divided into smaller blocks

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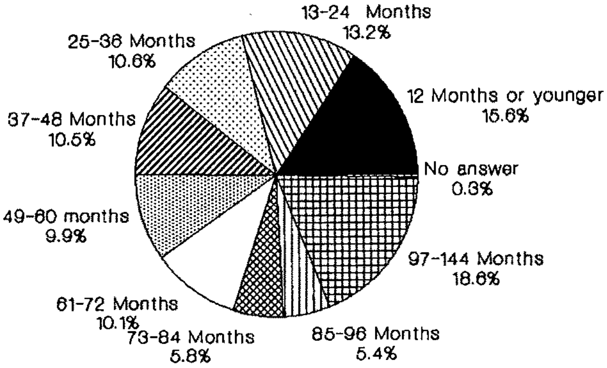

Fig 1. Ages of children in study.

of approximately equal size; and a sample of each block was randomly selected. Within each block selected, a systematic process was used whereby a random starting point was chosen and a predetermined zigzag route followed, so that we called at every other household encountered. There were two survey teams; each included an otolaryngology specialist, a nurse, a social worker, and a field supervisor. The team established whether there were any children in the house, and if so, after obtaining permission from the family, proceeded to clinical examination of the child and completed the relevant questionnaire with the parent (mainly the mother), which included, among many other variables, the age, sex, birth weight, parents' relationship, family history of deafness, complaints of hearing or speech defect, history of exposure to the known causative factors of childhood deafness, and perinatal history with special emphasis on the presence or absence of the risk factors for hearing impairment. Each child was assigned a code number and the questionnaires were subsequently designated by code number only. A series of investigations were routinely undertaken. All of the children were screened and those with a suspected history of deafness or exposure to the known risk factors were subjected to a pediatric and otological examination. Hospital records were obtained on all the children to determine if any significant abnormality had occurred in pregnancy or during or after birth.

As part of the testing done, pure tone audiometry or auditory evoked response was done in the hospital to determine the extent and type of any hearing impairment. The auditory brain stem evoked response was used only in children who were unable to cooperate. These tests were done by a qualified audiologist in the audiology unit. A pure tone audiologic evaluation for young children used the methods of limits by air conduction of 250 to 8,000 Hz and bone conduction of 500 to 4,000 Hz. The standard technique was employed for masking air and bone conductions. Brain stem auditory evoked potential waves were elicited by click stimuli generated by a 100-millisecond square-wave electrical pulse through earphones.

TABLE 1. "AT-RISK" AND "NORMAL" CHILDREN BY DEMOGRAPHIC CHARACTERISTICS

	Results of Examination	
Demographic	*At Risk*	*Normal*
Sex		
Boys	10.76 (691)	89.24 (2,841)
Girls	8.79 (565)	91.21 (2,324)
Age		
12 mo or younger	8.3	91.7
13-24 mo	9.2	90.8
25-36 mo	10.5	89.5
37-48 mo	12.0	88.0
49-60 mo	12.3	87.7
61-72 mo	11.3	88.7
73-84 mo	11.7	88.3
85-96 mo	8.4	91.6
97-144 mo	6.8	93.2

Data are percentages (row percentage = 100). In parentheses are numbers of children.

Recordings were made at a stimulus of 60 dB above hearing threshold level (dB HL). Three electrodes were placed at three points on the head, ie, left and right mastoid on both earlobes, and at the central zone. Recordings were made routinely between the vertex and the ipsilateral ear. Impedance audiometry in the form of tympanogram, acoustic threshold, and acoustic reflex decay was done in some of the children.

By means of the general and otolaryngological examinations, the series of investigations, and the risk factors, all the sampled children were examined to identify those children at risk of hearing impairment. The effect of hearing disorders among siblings and family members on the prevalence rate of hearing impairment was also assessed.

RESULTS

Among the total number of Saudi infants and children involved in the study, 1,256 (19.56%) of them were judged to be "at risk" of hearing impairment, while 5,165 (80.44%) of them were judged to be normal (not at risk).

The sample survey consisted of a total of 6,421 Saudi children: 55% (3,532) were boys and 45% (2,889) were girls. (The latest census in Saudi Arabia showed that 51% are males and 49% are females.)

The ages ranged from 2 months to 12 years of age, with an average of 56.67 months old. The age distribution of the sampled children is shown in Fig 1.

TABLE 2. AUDIOLOGIC FINDINGS

Hearing Loss	*No. of Children*
Conductive	
15-25 dB	112
25-35 dB	168
35-50 dB	46
Severe to profound SNHL	
Bilateral	155
Unilateral	13

SNHL — sensorineural hearing loss.

Altogether, about half of them (49.9%) were under the age of 4 years, while 20% were between 4 and 6 years old, and 29.8% were older than 6 years of age. Only 0.3% of the sampled children did not give their accurate age at the time of the study.

Table 1 shows the two groups of sampled Saudi children — "at risk" and "normal" — by demographic characteristics. With regard to the sex of the children, the present sample indicated that 10.76% of the at-risk children were boys (691) and 8.79% were girls (565).

The number of infants and children with hearing impairment was 494: 168 with sensorineural hearing loss, and 326 with conductive hearing loss. Out of the 168 with sensorineural hearing loss, 111 had bilateral severe to profound hearing loss that was congenital and hereditary (1.73% of the total number) and 57 had loss that was acquired. The audiologic findings are shown in Table 2.

With the exception of 1.7% of the sample, the other children had at least 1 brother or sister. About 17.5% of them had 1 or 2 siblings, 28.0% had 3 or 4 siblings, 46.6% had 5 or more siblings, and in 6.2% the number of siblings was unknown (Fig 2). The average number of siblings in this sample was 5.39, with a range from 1 to 13. Siblings older than 12 years of age were not included among the total number surveyed.

Two hundred fifty-seven children had a positive family history of deafness (0.7% mother, 0.9% father, 2.3% siblings, 1.1% uncles, 0.5% aunts, and 0.8% cousins), ie, 4% of the total sample surveyed. Those with actual hearing impairment were 72 children. The number of children with a positive family history but not deaf was 185; 35 of them were also considered at risk because they were exposed to other risk factors, namely, meningitis and low birth weight.

Our results show that the rate of hearing impairment among children whose siblings have hearing problems is 24.8%, compared to 7.7% among the total children surveyed ($p < .001$) and 7.3% among children with normal-hearing siblings.

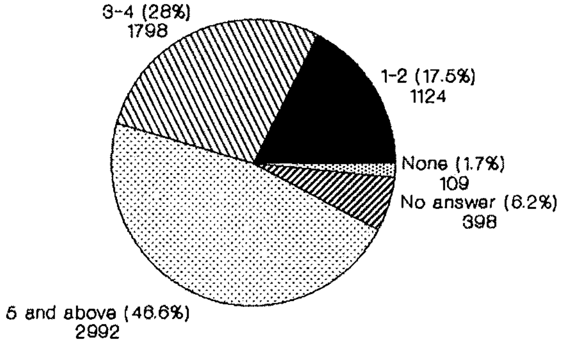

Fig 2. Numbers of siblings.

The total number of siblings of the hearing-impaired children was 511. One hundred seventy-three siblings (35%) of the children with hearing impairment showed hearing problems, as compared to 7.7% of the total sample ($p < .001$).

Regarding the age of the children, relatively higher at-risk rates were found among children between the ages of 2 and 7 years old. All other age groups had at-risk rates below the total sample's average rate.

The prevalence of consanguineous marriages among parents of all the children surveyed was found to be 22.1% first cousins and 23% second cousins. Consanguinity among children with hearing impairment was found to be 46%, compared to 36.4% among normal-hearing children. Consanguineous marriage is encouraged as part of the social customs in Saudi Arabia.

DISCUSSION

The occurrence of hearing impairment within one or more of the family members presents a diagnostic problem when there are no physical features present to enable identification of a syndrome. Investigation should be carried out to identify environmental factors that may have caused the defect or accompanying features that may signify a genetic condition for which the mode of inheritance is known.

In spite of vigorous investigation by the means currently available, there are an appreciable number of children for whom no causative factors can be identified in etiologic investigation.[4-6]

In this paper, a large-scale epidemiologic study to identify children at risk of hearing impairment was carried out. One of the purposes of this study was to assess the effect of hearing and speech disorders among siblings and family members on the prevalence rate of hearing impairment. Our results showed that whenever one's siblings have hearing or speech problems, one's probability of having these problems increases accordingly. The results did not show any statistical relationship with whether their mothers or fathers or aunts had hearing problems.

The prevalence of consanguineous mating is dictated to a large extent by culture, tradition, religion, and civil law. It is extremely common in some parts of the world, like southern India,[7] because of traditions; while it is not common in other parts, such as North America, because the low birth rate reduced the number of relatives available and also because some consanguineous marriages are prohibited by law or religion. This study showed that consanguinity is widely practiced in this Saudi community: first-cousin marriage was present in 22.1% of the parents of the children, and more distant consanguinity (second cousins) was present in 23%. This high frequency of consanguinity is due to social customs, the practice of arranged marriage within the families, and unawareness of the public about the adverse genetic effects of such practices. While marriages within certain degrees of kindred such as uncle-niece mating are prohibited on religious grounds in Saudi Arabia, other consanguineous marriages, such as first cousins mating, are encouraged as part of the social customs.[8]

The majority of hereditary hearing loss (70% to 80%) is inherited by a recessive single gene.[9] Consanguineous marriage increases the risk of autosomal recessive inheritance. It also increases the risk of transmission of polygenic (multifactorial) inheritance. This uncommon type of inheritance is not fully understood, but it is postulated that multiple genes contribute to the disease and that each individual has a threshold above which the abnormality will be manifest.[10] A further point is that for multifactorial inheritance, the risk to subsequent siblings is higher when the parents are consanguineous than when they are unrelated.[11]

In the event that mating of the individual with an isolated hearing loss takes place with an affected spouse, the risk to the offspring would depend on whether the hearing loss of the affected spouse was genetic or not.

Newton[12] has reported that according to Mendel's law the actual risk for a sibling of a child with an isolated hearing loss to have a sensorineural hearing loss is 1 in 4 if the loss is the result of an autosomal recessive trait, and there is a negligible risk if there is an environmental cause. If a sibling in an isolated case has a hearing defect, then autosomal recessive inheritance is indicated in most instances because of the low likelihood of a second new dominant mutation. A very low or negligible risk would also apply if the loss was the result of a dominant mutation, and a risk estimate of 1 in 9 has been indicated by segregation analysis for Newton's study groups as a whole. This is less than the risk of 1 in 6 suggested by Stevenson and Davison[13] and Harper.[14] The risk for a sibling of a patient with deafness has been examined by using the method of segregation analysis employed by Taylor et al.[4] Segregation analysis is based on Mendel's first law, in which the ratio of normal homozygote dominants to heterozygote carriers to affected homozygote recessives is 1:2:1.[12]

Hearing loss is likely to be found if there is a family member with a sensorineural hearing loss, and can be predicted more readily in those instances in which the hearing loss is recessive in origin.

Gene expressivity has been described as varying little within sibships.[15] This indicates that the degree of hearing loss likely to be found if any sibling of a child with isolated hearing loss is hearing-impaired would be similar to that of the first affected child. Where the loss in the latter follows a deteriorating course, then a similar progression would be expected in a sibling.

Our study has shown that children exposed to a greater risk of hearing impairment are those who have hearing-impaired siblings. Those children and their family members should be followed closely to identify as early as possible the hearing-impaired child in order to provide him or her with proper medical care and follow-up.

In summary, those children who had family members with hearing and speech problems were at higher risk compared to their counterparts regarding hearing impairment.

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Ann Otol Rhinol Laryngol 105:1996

EUSTACHIAN TUBE FUNCTION AND MIDDLE EAR BAROTRAUMA ASSOCIATED WITH EXTREMES IN ATMOSPHERIC PRESSURE

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Eustachian tube (ET) function was studied by means of sonotubometry and tubotympano-aerodynamography (TTAG) prior to and following exposure to hypobaric or hyperbaric conditions. Forty normal adults were subjected to hypobaric pressure. Fifty adults who underwent hyperbaric oxygen (HBO) therapy also were studied. Following hypobaric exposure, 14 of 80 ears (17.5%) exhibited middle ear barotrauma. Following hyperbaric exposure, 34 of 100 ears (34%) exhibited middle ear barotrauma. Dysfunction of the ET, characterized by altered active and passive opening capacity, was more prevalent following exposure to extremes in atmospheric pressure compared to baseline. The ET function, which was impaired after the first HBO treatment, improved gradually over the next 2 hours. Overall, however, ET function was worse after the seventh treatment. The patients who developed barotrauma exhibited worse ET function prior to hypobaric or hyperbaric exposure. Thus, abnormal ET function can be used to predict middle ear barotrauma prior to exposure to hypobaric or hyperbaric atmospheric pressure.

KEY WORDS — atmosphere exposure chamber, atmospheric pressure, barotrauma, eustachian tube, hyperbaric oxygenation, middle ear.

INTRODUCTION

Rapid changes in atmospheric pressure can be experienced as a fullness in the ear. These changes often are encountered during travel, particularly by airplane, and during underwater diving or hyperbaric oxygen (HBO) therapy. When there is a sufficient change in the ambient atmospheric pressure coupled with an inability to equalize the pressure in the middle ear with the atmosphere (by opening the eustachian tube [ET]), aural barotrauma occurs.[1-11] This issue has become more clinically significant as people spend more time in different atmospheric environments. A knowledge of the sequelae of maladjusted pressure in the middle ear is essential for all otologists in the era of air transport, and with exploration of the undersea world.[12]

Continuous administration of pure oxygen under hyperbaric conditions (HBO therapy) increases the dissolved oxygen content in the blood out of proportion to the increase in environmental pressure. Thus, HBO can be used in the treatment of acute, chronic, generalized, or localized hypoxic disorders. It can be anticipated that remarkable results will be achieved with this technique, which is currently used in the treatment of sudden deafness, idiopathic bilateral sensorineural hearing loss, and Bell's palsy.[13,14] Of considerable significance, therefore, is the fact that patients undergoing this therapy often complain of ear pain and fullness that can be transient or long-lasting.

We objectively measured changes in ET function in adults prior to and following exposure to extremes in atmospheric pressure in a low-pressure chamber or a hyperbaric oxygen chamber using sonotubometry with swallowing and the impedance method of tubotympano-aerodynamography (TTAG) with Valsalva's maneuver. This study was designed to determine the prevalence of middle ear and ET dysfunction with rapid changes in atmospheric pressure, and to identify patient groups at risk for developing middle ear barotrauma.

MATERIALS AND METHODS

Influence of Hypobaric Exposure. A group of 40 normal, healthy adults (80 ears) were studied. There were 33 men and 7 women. Their ages ranged from 20 to 47 years (mean age 29 years). The subjects were screened by history and physical examination for the presence of nasal or ear disease by a team of otolaryngologists. Prior to and following exposure to hypobaric pressure in the hypobaric chamber of the Research Institute of Environmental Medicine, Nagoya University, ET function tests were performed, and the tympanic membrane was examined. The pressure was reduced (ascent) and applied (descent) at a rate of 0.015 atmospheres absolute (ATA) per minute (Fig 1). In most commercial aircraft, cabin pressure is reduced at a rate of 0.015 ATA/min (at an

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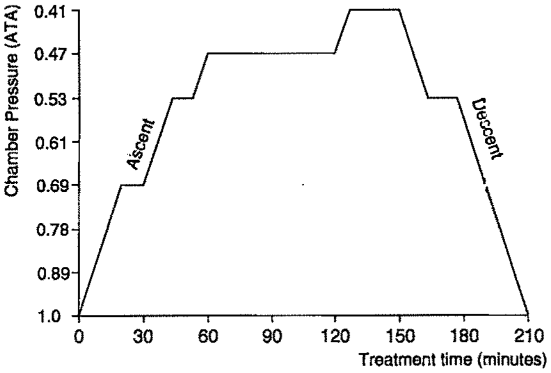

Fig 1. Decompression pattern in hypobaric chamber.

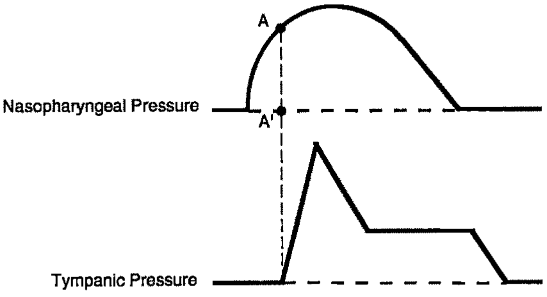

Fig 2. Schematic representation of nasopharyngeal pressure and impedance in tympanic membrane indicating measurement points for determining tubal opening pressure, which corresponds to minimum nasopharyngeal pressure for ventilation (A–A').

ascent of 150 m/min), and increased at a rate of 0.009 ATA/min (at a descent of 90 m/min). The subjects were instructed to perform Valsalva's maneuver, or to swallow when they experienced ear fullness or otalgia in the hypobaric chamber. Pure oxygen was administered intermittently if necessary.

The JK-04 ET function analyzer (RION, Tokyo, Japan) was used to perform sonotubometry and impedance TTAG. Eustachian tube function can be evaluated by passive and active opening capacity; this is the ability of the ET to open actively by swallowing and to open passively by increased nasopharyngeal pressure induced by Valsalva's maneuver.[15-18] Sonotubometry is a method of assessing the active opening capacity of the ET. Sound is presented into the nares and detected by means of a microphone placed in the external auditory canal.[19-22] The subjects perform a sequence of two dry swallows over a period of 10 seconds. If the ET opens, a brief interval of increased sound transmission is detected. Impedance TTAG is performed by means of the simultaneous recording of impedance in the tympanic membrane and nasopharyngeal pressure. The passive opening capacity of the ET with Valsalva's maneuver can be detected in a wave pattern of impedance changes recorded from the external ear canal.[23-26] The tubal opening pressure was recorded, and the mean values were calculated (Fig 2).

Influence of Repetitive Hyperbaric Exposure. Fifty adolescent or adult patients (100 ears; 26 males and 24 females) underwent HBO therapy for sudden deafness, idiopathic bilateral sensorineural hearing loss, or Bell's palsy from 1993 to 1994 at the Hospital of Nagoya University. Their ages ranged from 13 to 69 years (mean 44 years). Only patients without symptoms or signs of middle ear disease were included. The HBO therapy was performed in a large chamber that can accommodate 10 people simultaneously in order to eliminate the risk of explosion and to ensure safe treatment. The HBO therapy was administered at a pressure of 2 ATA for 60 minutes. Compression to 2 ATA occurred in 7 minutes, with decompression in 13 minutes (Fig 3). Patients received one treatment per day. The number of HBO treatments ranged from 9 to 24 (mean 16). All patients were routinely instructed to equalize middle ear pressure during treatment with Valsalva's maneuver or swallowing. Compression or decompression was suspended temporarily if otalgia occurred. If complications were too severe to continue treatment, HBO therapy was discontinued for several days. The tympanic membrane was examined prior to each treatment. The JK-04 was used to perform sonotubometry and impedance TTAG. Eustachian tube function testing was performed prior to treatment and following the first, seventh, and last HBO sessions. After the first HBO session, measurements were made immediately after decompression, and 1 and 2 hours later.

Statistical analysis was performed by the χ^2 test and Student's *t*-test. A p value of $< .05$ was considered significant.

RESULTS

Hypobaric Exposure. At the time of enrollment in the study, all 40 subjects had normal tympanic membranes. Most subjects developed a sensation of full-

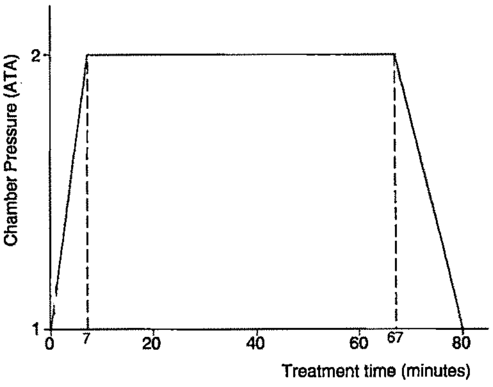

Fig 3. Pressurization pattern during hyperbaric oxygen therapy.

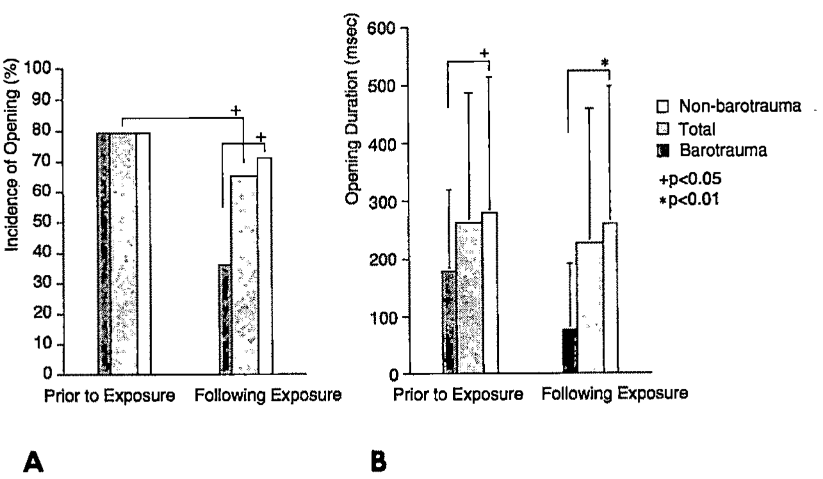

Fig 4. Active eustachian tube (ET) opening assessed by sonotubometry following hypobaric exposure. A) Incidence of ET opening. B) Duration of ET opening.

ness in their ears during decompression and compression. Otalgia during compression was reported by 10 subjects. Congestion or hemorrhage of the tympanic membrane was noted in 14 (17.5%) of the 80 ears after treatment. None of the patients developed inner ear barotrauma.

The incidence of active opening of the ET was significantly lower, and the average duration of opening was slightly shorter, following hypobaric exposure (Fig 4). In the group that developed barotrauma the postexposure incidence of active opening was significantly lower than in the group that did not develop barotrauma (Fig 4A). The duration of opening in the barotrauma group was significantly shorter than in the non-barotrauma group, both prior to and following hypobaric exposure (Fig 4B).

The incidence of passive ET opening assessed by impedance TTAG was significantly lower following hypobaric exposure (Fig 5A). In addition, the average opening pressure was slightly elevated (Fig 5B). In the group that developed barotrauma, the postexposure incidence of passive opening, and the preexposure and postexposure opening pressures, were not significantly different from those in the non-barotrauma group.

Repetitive Hyperbaric Exposure. Most patients developed a sensation of fullness in their ears during HBO treatment. Congestion or hemorrhage of the tympanic membrane, or fluid or blood in the tympanic cavity, was noted in 34 of the 100 ears (34%). Middle ear barotrauma occurred following 6 (SD = 3) sessions. No patients developed inner ear barotrauma.

The incidence of active opening of the ET was significantly lower 1 hour following the first hyperbaric exposure, and following the seventh session, when compared to baseline (Fig 6A). There was no significant difference in the incidence of active opening prior to exposure and at the termination of treatment. The group that developed barotrauma exhibited a significantly lower incidence of ET opening compared to the group that did not develop barotrauma throughout the course of HBO therapy. The average duration of opening (Fig 6B), incidence of passive ET opening (Fig 6C), and opening pressure (Fig 6D) followed a similar pattern following hyperbaric exposure.

The patients were classified into three groups based on the results of ET function testing prior to

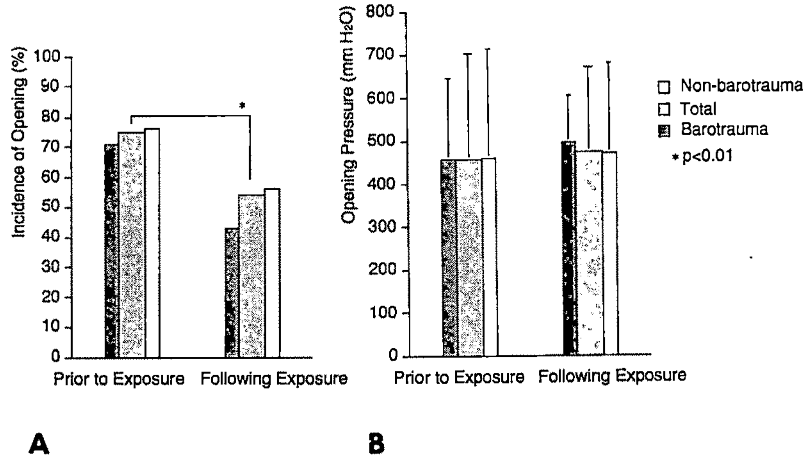

Fig 5. Passive ET opening assessed by impedance tubotympano-aerodynamography following hypobaric exposure. A) Incidence of ET opening. B) Tubal opening pressure. If ET failed to open, maximum nasopharyngeal pressure recorded was used to represent opening pressure for convenience of comparison.

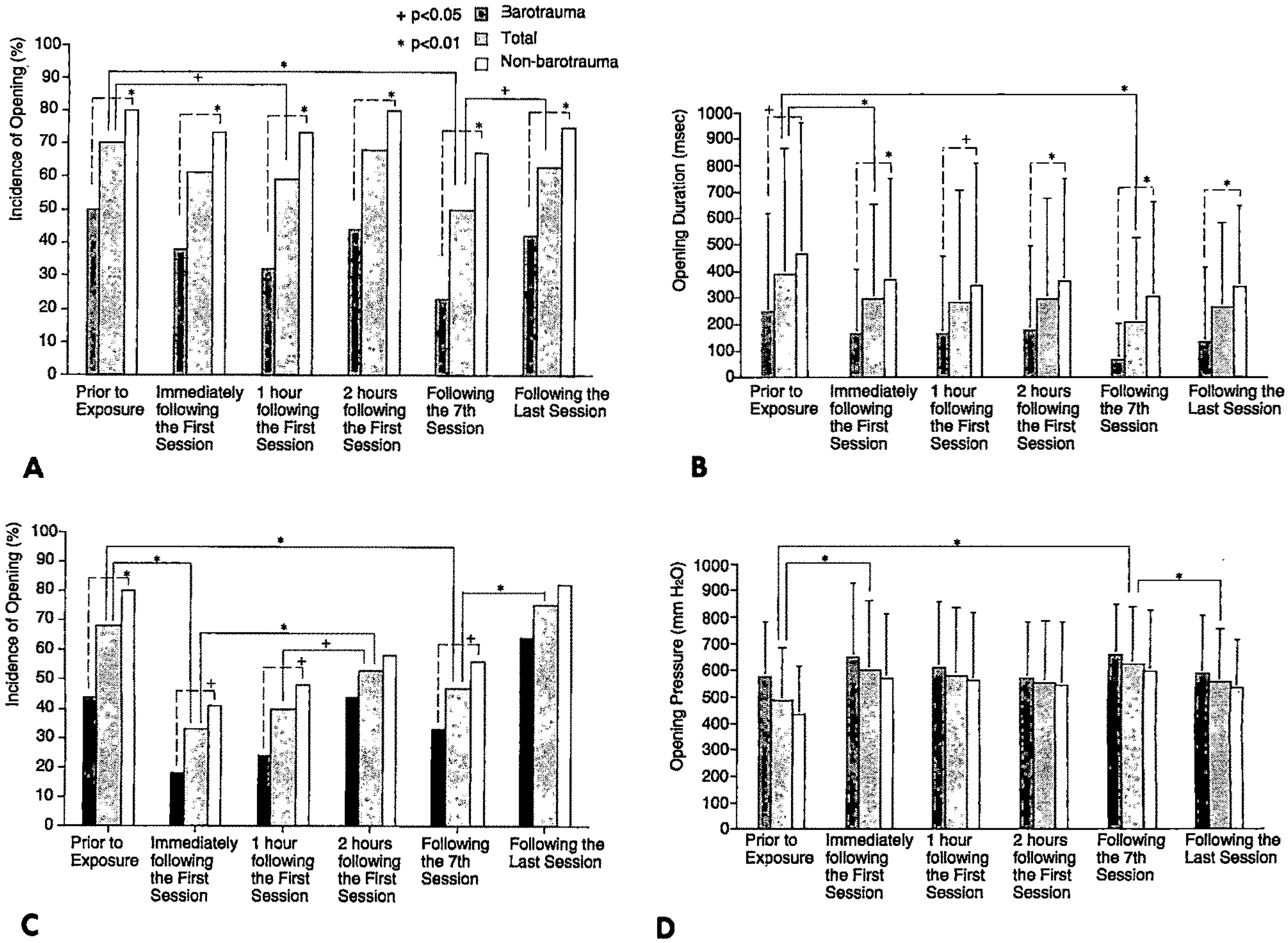

Fig 6. Findings following hyperbaric exposure. A) Incidence of active ET opening as assessed by sonotubometry. B) Duration of ET opening as assessed by sonotubometry. C) Incidence of passive ET opening as assessed by tubotympano-aerodynamography. D) Tubal opening pressure measured by tubotympano-aerodynamography. If ET failed to open, maximum nasopharyngeal pressure recorded was used to represent opening pressure for convenience of comparison.

HBO treatment (Fig 7). In the first group ("good") there were 49 ears demonstrating normal ET function, 7 of which (14%) developed barotrauma. The second group ("bad") consisted of 40 ears exhibiting a negative examination by sonotubometry or impedance TTAG. Barotrauma was noted in 18 ears (45%) in this group. Nine of 11 ears (82%) in the third group (negative on both tests) developed barotrauma.

DISCUSSION

The equality of air pressure across the tympanic membrane is maintained by the ET, which opens and permits the passage of gas.[12] The tube acts like a flutter valve between the pharynx and the middle ear (Fig 8), allowing the exit of air from the middle ear more readily than it allows admission from the nasopharynx.[27] In decompression, the increased pressure of the gas contained in the middle ear will passively open the ET. The combination of passive tubal opening and the normal action of swallowing (active opening) allows decompression with little or no difficulty. In compression, a completely different mechanism is involved. The majority of cases of aerotitis media occur at this time. The relative negative pressure in the middle ear tightens the closure of the ET. Thus, the ET must be opened by swallowing or therapeutic inflation (Valsalva's maneuver).[12,27] It is generally agreed that aural barotrauma occurs as a direct result of failure to equalize differences in pressure between the middle ear and the atmosphere.[1,2,8] This is dependent on ET function, and occurs upon failure of the ET to open voluntarily, or because of the inability of the ET to open when it is exposed to changes in barometric pressure.

Various methods have been employed for the examination of ET function.[26,28] We tested ET function using sonotubometry to assess active opening capacity and impedance TTAG to assess passive opening capacity. These methods are reliable and relatively easy to perform with the JK-04 ET function analyzer. The equipment is now commercially available and is becoming a routine clinical tool in Japan.

ET function	Good	Bad	Worse
Barotrauma	7/49 (14%)	18/40 (45%)	9/11 (82%)

+$p<0.05$, *$p<0.01$

Fig 7. Incidence of middle ear barotrauma according to results of ET function tests performed prior to exposure to hyperbaric pressure.

In this study, ET dysfunction, characterized by altered active and passive opening capacity, was observed following hypobaric exposure. In the group that developed barotrauma, the duration of opening was shorter both prior to and following hypobaric exposure, and the postexposure incidence of active opening was lower than in the group that did not develop barotrauma.

Following repetitive hyperbaric exposure during HBO therapy, passive and active ET dysfunction was noted. The marginal improvement in function at the end of the course of treatment was likely due to the more effective utilization of equalization techniques as the patients became more experienced. Middle ear barotrauma was noted in most of the patients after the seventh day of HBO therapy, which corresponded to the highest incidence of ET dysfunction. The group that developed barotrauma exhibited worse ET function throughout the course of HBO therapy than the non-barotrauma group. In the patients with abnormal active and passive opening prior to HBO therapy, the incidence of barotrauma was greatest. Myringotomy or placement of ventilation tubes might prevent barotrauma in these high-risk patients. None of the patients required discontinuation of HBO therapy because of middle ear barotrauma. Although most cases of barotrauma are transient, it is important to predict its occurrence to decrease the incidence of this complication.

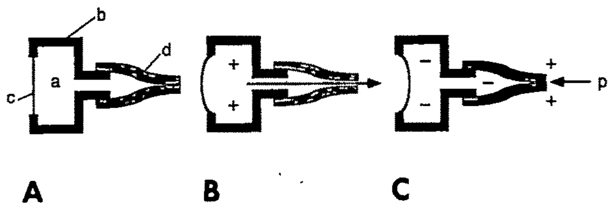

Fig 8. Diagram showing pressures in tympanic cavity (a) and action of ET (d) during flight. b — bony capsule of tympanic cavity, c — tympanic membrane, p — compressed air in nasopharynx. A) Normal condition. B) Decompression. C) Recompression.

In conclusion, ET function worsens following exposure to extremes in atmospheric pressure. Dysfunction of the ET predisposes to middle ear barotrauma. Sonotubometry and impedance TTAG are valid indicators of ET function that can be used to identify groups at risk for developing middle ear barotrauma following exposure to hypobaric or repetitive hyperbaric atmospheric pressure.

ACKNOWLEDGMENTS — The authors gratefully acknowledge the staff of the Department of Hyperbaric Medicine and the Research Institute of Environmental Medicine, Nagoya University, for their assistance.

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LANGUAGE DEVELOPMENT IN COCHLEAR IMPLANTED CHILDREN

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Ann Otol Rhinol Laryngol 105:1996

PATTERNS OF PERSISTENT OTITIS MEDIA IN THE FIRST YEAR OF LIFE IN ABORIGINAL AND NON-ABORIGINAL INFANTS

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Australian Aboriginal infants experience very early otitis media (OM). A previous study reported that OM with effusion (OME) or acute OM (AOM) was observed in the first 8 weeks of life in 95% of 22 Aboriginal infants, but that OME was seen in only 30% of 10 non-Aboriginal infants. Tympanic membrane perforation was reported for 1 Aboriginal subject at 8 weeks of age. This requires further investigation, because early OM onset has been demonstrated in non-Aboriginal groups to increase the risk of chronic and persistent ear disease in later childhood. This prospective study used otoscopy and tympanometry to describe the course of OM in infants examined repeatedly from soon after birth. Disease course was described in two ways, based upon earlier findings from other studies of Aboriginal schoolchildren. First, patterns of disease in the first year were identified; non-Aboriginal infants had occasional episodes of OME or AOM from which they recovered spontaneously, usually within 1 month; Aboriginal infants had persistent AOM, OME, or tympanic membrane perforation with discharge that rarely, if ever, resolved to normal. Second, conditional probabilities were calculated for ear state transitions at consecutive ear examinations, and a model of the course of OM was proposed for the Aboriginal infants. Results also suggested that binaural patterns of chronic OM described previously in Aboriginal schoolchildren may already be established in the first year of life. These findings will help service providers determine when to intervene to avoid the chronic consequences of early OM.

KEY WORDS — diagnosis, indigenous populations, infants, otitis media.

INTRODUCTION

Current knowledge about the natural history of middle ear disease in Australian Aborigines derives mainly from studies of school-age children, reflecting appropriate concern that 50% to 80% of Aboriginal schoolchildren are estimated to be affected by chronic otitis media (OM) and concomitant conductive hearing loss.[1,2] Much less is known about the early onset and natural history of Aboriginal children's middle ear disease in infancy and the preschool years. This knowledge is necessary for planning and monitoring of community health promotion and middle ear disease prevention programs and for effective early detection and medical management, especially since it is well known in non-Aboriginal populations that children with early OM are at high risk for repeated disease throughout childhood.[3,4]

In school-age Aboriginal children, the course of OM has been described from cross-sectional surveys, but the early, acute phases of the disease have not been well described. For instance, Dugdale et al[5] reported different prevalence rates for different middle ear conditions in childhood and adolescence. The proportions of normal ears and of ears with scarred tympanic membranes (TMs) increased throughout middle childhood, while the proportions of TMs with perforations or that were chronically inflamed decreased. These authors attributed the different prevalence rates in their cross-sectional survey to gradual and spontaneous improvement in ear health throughout childhood and adolescence.

From results of a longitudinal study of Aboriginal children ages 6 through 15 years, McCafferty et al[6] proposed five patterns of ear health variation in school-age children who had been examined at least five times in 9 years. The patterns described children with consistently perforated TMs (pattern I), with perforations almost always but with occasional intact TMs (pattern II), with initial perforations but later intact TMs (pattern III), with intact but abnormal drums and likely effusion (pattern IV), and those with usually intact TMs and no significant otoscopic abnormality (pattern V). This study also demonstrated a gradual improvement in middle ear health with age, but it indicated that many subjects continued to experience chronic OM into adolescence according to their established pattern.

Neither of the above studies examined a sufficient number of infants to describe patterns in their middle ear condition, and so the disease course they described reflected predominantly chronic conditions well after onset. Other studies have proposed models of middle ear disease onset and progression in the acute phase from cross-sectional data. For instance, Foreman[7] examined 436 ears in Australian Aboriginal infants and young children in three remote north-

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ern communities. The prevalence of TM perforation was 11% at age 0 to 6 months, 43% at 7 to 12 months, and 30% at 13 to 24 months, and there were signs of middle ear disease in the intact TMs of infants less than 6 months: 20% of TMs were inflamed and 35% were dull. Foreman hypothesized a natural history of early OM based on a cross-tabulation of right and left ear states; perforation of a TM was significantly associated with reddening of an immobile TM in the contralateral ear, suggesting that perforation was usually preceded by (subacute) inflammation. A longitudinal study of infants in another northern Australian Aboriginal community observed only TM perforation events and found that many infants had their first perforation before age 12 months, and that the time of the first perforation was age-specific, with peak incidences at around 18 and 50 weeks.[8]

The present study, therefore, sought to examine Aboriginal and non-Aboriginal infants prospectively from soon after birth and to describe middle ear state transitions during the first year. Normal middle ear status was observed in most subjects in the neonatal period, and OM commenced in some infants in the first 8 weeks as previously reported.[9] The present study aimed to test the hypotheses that 1) the course of early OM is different in Aboriginal infants and non-Aboriginal infants, 2) different patterns of ear disease may be defined that describe the course of disease in Aboriginal infants, and 3) the patterns of disease in Aboriginal infants are similar to patterns of chronic disease reported previously in older Aboriginal children.

Parallel microbiologic investigation of bacterial nasopharyngeal colonization in these infants was carried out and reported elsewhere.[10]

SUBJECTS

Two groups of subjects were enrolled in a longitudinal audiologic study of OM in infants, conducted between January 1992 and July 1993. Forty-one Aboriginal infants, born between January 1992 and June 1993 to Aboriginal mothers usually resident in a northern Australian tropical island community 70 km north of Darwin, were recruited. A further 17 non-Aboriginal infants (10 male, 7 female), born between June 1992 and May 1993 to non-Aboriginal mothers residing in Darwin, were recruited for comparison with the Aboriginal group. This paper describes data for two overlapping subsets of subjects: first, those examined at least three times in the first 6 months, including one examination after the neonatal period (26 Aboriginal and 10 non-Aboriginal infants), and second, those with two or more examinations 15 to 30 days apart (32 Aboriginal and 11 non-Aboriginal infants).

PROCEDURES

Otoscopy and tympanometry were performed by the first author, a clinically certified audiologist who was trained and validated in pneumatic otoscopy by an experienced pediatric otologist and who gained additional experience in examining neonates and infants during the 12 months preceding the study. Diagnostic agreement between the author and the otologist in a sample of study subjects was 81% with otoscopy alone, and 96% with otoscopy and tympanometry. All subjects were examined soon after birth in the Royal Darwin Hospital and then at approximately 4-week intervals throughout the study period, either at their home community health clinic, in the case of Aboriginal subjects, or at the hospital, in the case of non-Aboriginal subjects.

The external ear canal and the TM were examined with a Welch Allyn 3.5-V pneumatic otoscope with 2- to 3-mm, dark green specula. Ear canal contents, TM appearance (color, translucency, and position), and TM mobility in response to applied air pressure change were recorded. Tympanometry was performed on cooperative infants with intact TMs by means of a GSI-27A automatic admittance meter with a 226-Hz probe tone.

Caregivers were asked about the presence of signs and symptoms of ill health during the week preceding the examination: nasal discharge; tugging or rubbing at the ears; atypical behavior at home, such as irritability, lethargy, or poor sleeping; diarrhea or loss of appetite; and fever or other acute respiratory symptoms. Cautiously, acute OM (AOM) was diagnosed when at least two of these symptoms were present in association with a red, bulging TM.

The condition most likely to be confused with AOM was OM with effusion (OME), another pathologic state. None of the ears diagnosed with AOM had TM perforations or normal TM appearance or mobility.

Otoscopy and tympanometry data and clinical observations were combined and the middle ear status was coded: normal = normal middle ear; OME = otitis media with effusion; AOM = acute otitis media; perforation = TM perforation with discharge; and unknown = uncertain ear status.

The binaural health of each subject was described from their individual middle ear status data and based on the status of the poorer ear. Five binaural health categories were developed by using modifications of the categories proposed by McCafferty et al as shown in Table 1,[6] which compares the categories used by McCafferty et al and in the present study. If the mid-

TABLE 1. BINAURAL CATEGORIES

McCafferty et al[6] Aural Health Categories		*Present Study Binaural Categories*	
Category 1	Both ears with intact TMs, no otoscopic abnormality, middle ear pressures more positive than −200 daPa	= Category A	Both ears with normal mobility on otoscopy and/or tympanometry
Category 2	One or both ears with intact TMs with otoscopic abnormality and/or middle ear pressure more negative than −200 daPa; no perforations	≠ Category B	Either or both ears with OME, neither with AOM or perforation
Category 3	One or both ears with wet or dry perforations	= Category D	Either or both ears with TM perforation
Category 4	One or both ears with cholesteatoma	≠ Category C	Either or both ears with AOM, neither with perforation
		Category E	Both ears with unknown status

TM — tympanic membrane, daPa — decapascals, OME — otitis media with effusion, AOM — acute otitis media.

dle ear status was unknown in one ear, it was assumed to be the same as in the other ear. Because the subjects examined by McCafferty et al were mostly between 6 and 15 years old and experiencing chronic OM, the present study defined two different categories of binaural health as shown in Table 1: that is, category B when either or both ears showed OME, neither with AOM or perforation; and category C when either or both ears showed AOM, neither with perforation.

The course of disease in individual subjects was then depicted graphically, by using the approach of McCafferty et al.[6] For this, intercategory variations in binaural health in the first year were plotted for each subject who was examined three or more times in the first 6 months (26 Aboriginal subjects and 10 non-Aboriginal subjects). Again, because the subjects of McCafferty et al were older, their patterns did not record AOM (patterns III and IV in this study). This infant study did not record any subjects with early OM who recovered normal ear health within the observation period as seen in pattern III by McCafferty et al. Thus, five patterns described the course of OM in these samples; these are defined in Table 2,[6] and an example of each pattern is shown in Fig 1.

Finally, a model of the natural history of OM was developed that described the individual variations in disease course during the first year of life, based on the model proposed by McCafferty et al[6,11] for school-age Aboriginal children, as shown in Fig 2A.[11] This was done by cross-tabulating individuals' middle ear status at one examination with their ear status at the following examination 15 to 30 days later and by calculating conditional probabilities for each transition in status. These conditional probabilities and the ear states they revealed were then inserted to adjust the model to reflect current findings.

RESULTS

Ear States and Binaural Health Categories. Figure 3 shows the distribution of middle ear status diagnoses, while Fig 4 shows the age-specific prevalence of OM by age in months for all subjects examined in their first year of life. In each age group for which comparison was possible, there were more Aboriginal ears with a diagnosis of OM.

Patterns of Ear Health Variation. Data from 36 subjects who were examined at least three times during the first 6 months were evaluated for binaural health variations during their first year. Each subject was allocated to one of five patterns of longitudinal variation in binaural health after McCafferty et al[6] as defined in Table 2. Table 3 shows the distributions of binaural health patterns for the Aboriginal and non-Aboriginal groups. A comparison of the two groups showed a distinct trend toward more chronic disease in the Aboriginal cohort. Pattern V, which represented normal ear health throughout all or most of the

TABLE 2. PATTERNS OF LONGITUDINAL CHANGE IN BINAURAL CATEGORIES

McCafferty et al[6]		*Present Study*	
Pattern I	Perforated TMs always or usually	= Pattern I	Persistent or frequent TM perforations
Pattern II	Perforations for most evaluations, occasional intact TMs	= Pattern II	Persistent or frequent OME or AOM and occasional perforations
Pattern III	Perforations in first 2 years of testing, TMs intact thereafter	≠ Pattern III	Persistent OME with or without episodes of AOM
Pattern IV	Persistent closed ear disease, occasional perforation or occasional normal ears	≠ Pattern IV	Persistent OME and/or AOM with occasional normal ears
Pattern V	Always or almost always normal ears, occasional closed ear disease	= Pattern V	Always or almost always normal ears, occasional OME

Abbreviations as in Table 1.

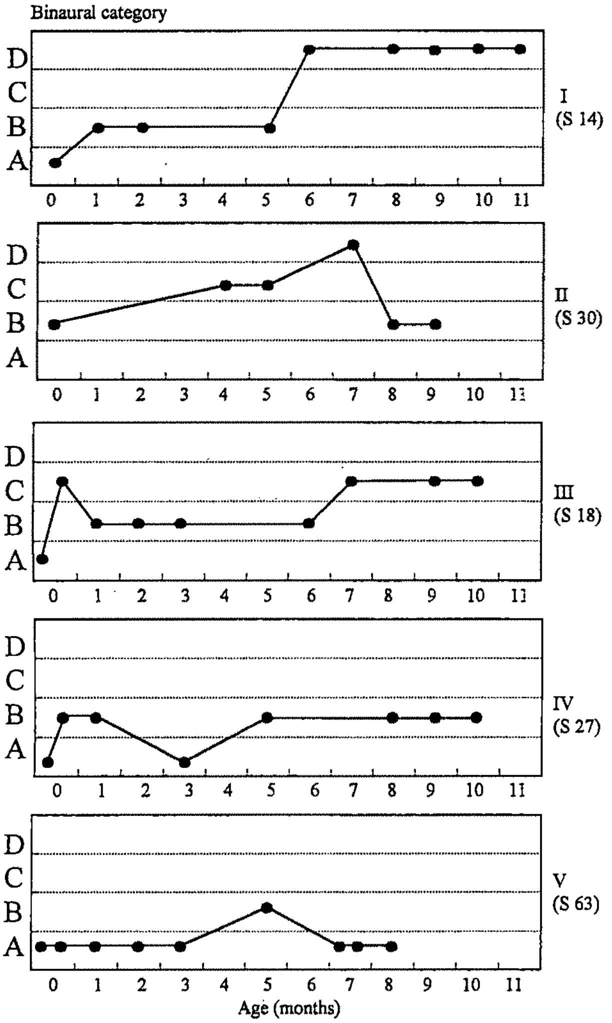
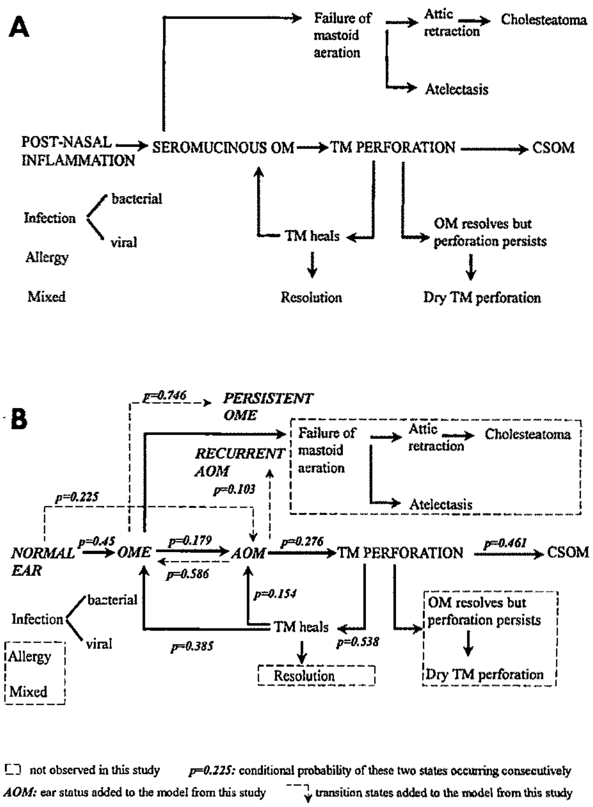


Fig 1. Sample patterns of binaural category variations in first year of life. See Tables 1 and 2 for key. S — subject number.

study period, did not describe any of the 26 Aboriginal infants, the majority of whom fitted patterns I, II, and III. Conversely, none of the 10 non-Aboriginal subjects evaluated by this method had persistent ear disease (patterns I and II). Of 26 Aboriginal subjects for whom longitudinal data were available, 9 (35%) had persistent perforations (pattern I), 5 (19%) had intermittent perforations that had closed by the following examination (pattern II), and none showed evidence of early perforations healing over time.

Natural History of Otitis Media. Conditional probabilities were calculated for ear state transitions in individual subjects, for both the Aboriginal and non-Aboriginal groups as shown in Tables 4 and 5, respectively. A comparison of data along and off the diagonal in the non-Aboriginal group revealed no significant variations in ear status at consecutive examinations ($\chi^2$1 = 0.09, p = .767), whereas ear status often changed between one examination and the next in the Aboriginal group ($\chi^2$1 = 5.34, p < .05), consistent with a chronic, fluctuating condition. In this group, the most likely outcome of any normal or abnormal middle ear state was OME. Furthermore, TM perforations were preceded by closed ear disease states (AOM more often than OME), and once the cycle of disease had been established, transitions occurred mostly between the different pathologic states, and middle ears rarely returned to normal. In the non-Aboriginal group, normal function was more likely, although some had occasional episodes of OME or AOM. Non-Aboriginal subjects with healthy middle ears were nearly three times as likely to remain so and, if they showed OME, were more than 22 times more likely than Aboriginal subjects to return to normal middle ear health. Moreover, the Aboriginal subjects were nearly 3 times more likely to develop OME and nearly 4 times more likely to develop AOM following an examination showing normal middle ear condition.



not observed in this study *p=0.225:* conditional probability of these two states occurring consecutively
AOM: ear status added to the model from this study transition states added to the model from this study

Fig 2. Flow diagrams depicting probable course of ear disease in Aboriginal children. A) Modified from McCafferty et al.[11] B) Further modifications proposed from present study.

This information was used to make additions and modifications to the model of natural history proposed by McCafferty et al,[6] including the division of the state "seromucinous OM" into the two states OME and AOM, and the addition of conditional probabilities for each transition recorded in Tables 4 and 5. Figure 2B shows the modified model based on

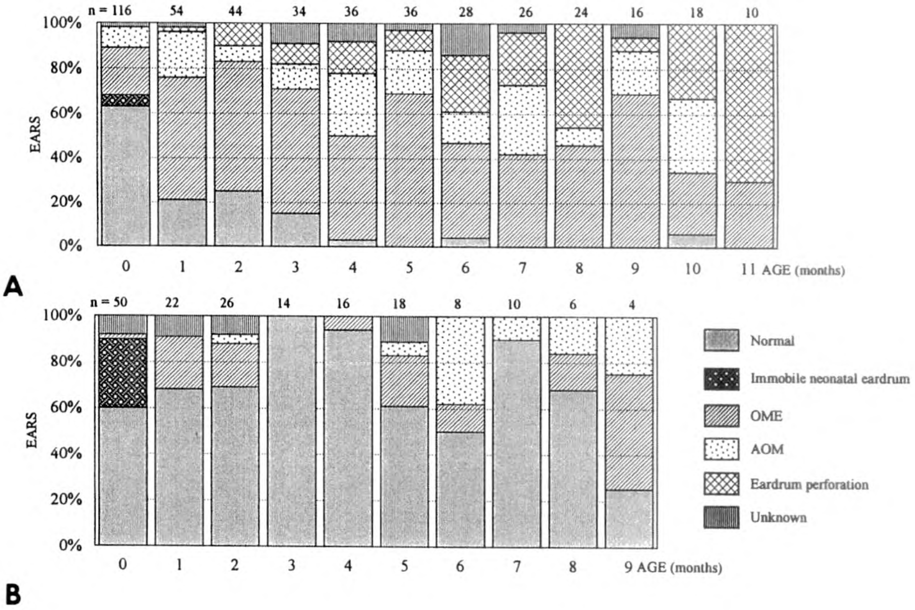


Fig 3. Frequency distribution of middle ear status by month in first year. n — number of ears examined. A) Aboriginal subjects. B) Non-Aboriginal subjects.

middle ear data from 32 of the Aboriginal infants.

DISCUSSION

This study demonstrated important differences between the Aboriginal and the non-Aboriginal infants in their early OM that parallel microbiological findings concerning their nasopharyngeal colonization with respiratory bacteria reported elsewhere.[10] Not only did OME and AOM appear earlier in the Aboriginal infants, but they also showed a distinct trend to more chronic conditions and more severe middle ear disease states. The non-Aboriginal infants frequently showed normal patterns of ear health or patterns with AOM and/or OME and subsequent resolution but no TM perforations, whereas Aboriginal infants rarely showed normal patterns following hospital discharge and frequently showed perforated TMs in addition to OME and/or AOM. For the non-Aboriginal children, these data are consistent with age-specific prevalence data for children elsewhere,[12,13] while the data for Aboriginal infants suggest that patterns reported by other investigators in school-age Aboriginal children may well be already established during early infancy.

There are important similarities and differences between these findings in Aboriginal infants and those in older, school-age Aboriginal children reported by McCafferty et al.[6] The groups were similar in that persistent middle ear disease was common in

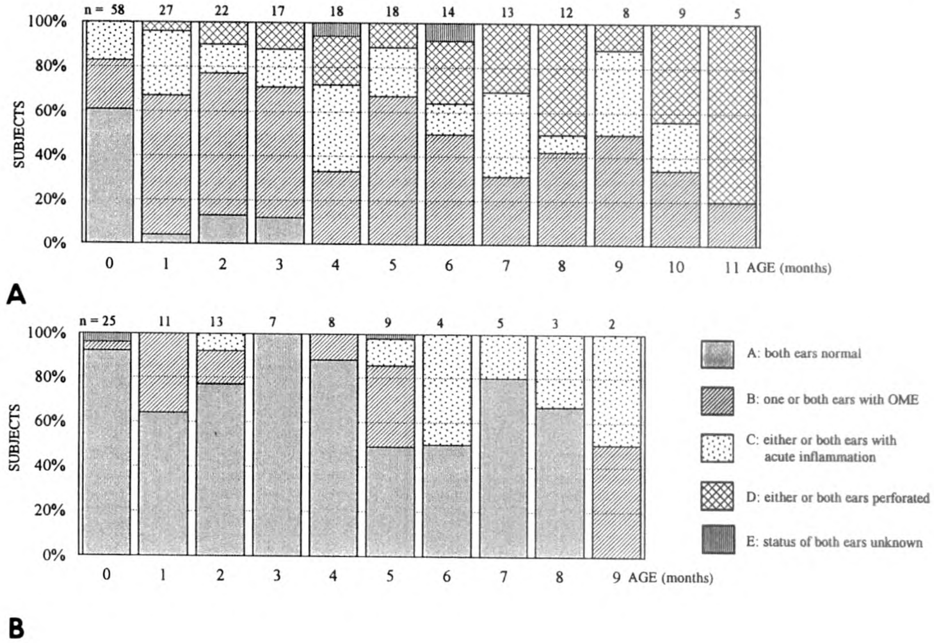


Fig 4. Age-specific prevalence of otitis media: binaural health category by month in first year. n — number of subjects. A) Aboriginal subjects. B) Non-Aboriginal subjects.

TABLE 3. DEFINITIONS AND DISTRIBUTIONS OF EAR HEALTH PATTERNS IN ALL SUBJECTS

Pattern	*Normal Ears*	*AOM or OME*	*Perf in TM*	*Abor*		*NonAb*	
				Ss	*%*	*Ss*	*%*
I	Never	Rarely	Mostly	9	35	0	0
II	Never	Rarely	Mostly	5	19	0	0
III	Never	Always	Never	11	42	0	0
IV	Rarely	Mostly	Never	1	4	1	10
V	Mostly	Rarely	Never	0	0	9	90
All				26	100	10	100

AOM — acute otitis media, OME — otitis media with effusion, Perf — perforation, TM — tympanic membrane, Abor — Aboriginal, NonAb — non-Aboriginal.

both and perforations with discharge occurred frequently (patterns I and II); in the infant group, 54% had persistent (35%) or intermittent (19%) TM perforations with discharge, and persistent OME was common in both this infant group and the older group studied by McCafferty et al. A significant difference was observed, in that the spontaneous improvement that occurred in some of the older group studied by McCafferty et al was not observed in the present, infant group. In the former group, 5.6% of children's middle ears followed pattern III, with early, persistent chronic suppurative OM leading to subsequent healing of the TM and aeration of the middle ear over the 9-year period, while no middle ears in the current infant sample returned to normal following early TM perforation, probably because of their youth and the short duration of the study compared to that by McCafferty et al.

There was a further important difference between the two studies in the proportions of subjects' middle ears that were classified as pattern V (consistently normal ears). Normal middle ears were observed repeatedly in 37% of the Queensland[6] and in 90% of non-Aboriginal infants in this study, but none at all in the present Aboriginal infant group. As with the previous difference, this is probably explained by the age difference between the two cohorts, because reducing OM prevalence with age through middle to late childhood has been frequently reported in other populations.[14,15] Studies of Aboriginal people have also reported reducing prevalence of OM with child age and apparent closure of TM perforations with a reduction in OM prevalence during early adolescence,[5,6,16] although rates of active OM in some adult Aboriginal groups are still well above those in comparable non-Aboriginal Australian groups.[17]

A final important difference between the present Aboriginal infant group and the school-age group reported by McCafferty et al[6] is the presence of AOM in the adjusted model depicting the probable course of OM in Aboriginal children shown in Fig 2B. While symptoms of AOM were frequently observed by infants' caregivers and the investigators in the present study, in the age group examined by McCafferty et al, AOM was not reported. This was possibly because the infant study sought specifically to observe middle ear condition during the first and early episodes of OM, whereas in older children, McCafferty et al observed ongoing conditions long after first onset, when AOM occurs; certainly, AOM has not been reported previously in literature concerning Aboriginal children beyond preschool age. Nevertheless, other significant, and possibly explanatory, differences between the groups cannot be discounted, including their testing by different examiners some 10 to 20 years apart, their different Aboriginal group origins, and their considerable geographic separation. (McCafferty's subjects resided in Cherbourg, in southern Queensland.)

Despite this, there were striking similarities in the binaural patterns of OM between these infants and the schoolchildren reported by McCafferty et al,[6] suggesting that the distinct patterns reported for Ab-

TABLE 4. TABLE OF CONDITIONAL PROBABILITIES OF TRANSITIONS, AND THEIR CONFIDENCE INTERVALS, BETWEEN EAR STATES FOR INDIVIDUAL ABORIGINAL EARS EXAMINED AT INTERVALS OF 15 TO 30 DAYS

	15 to 30 Days Later				
Earlier	*Normal*	*OME*	*AOM*	*Perf*	*Total*
Normal	0.3 0.17 - 0.467	0.45 0.296 - 0.613	0.225 0.114 - 0.388	0.025 0.001 - 0.147	0.268 (40)
OME	0.045 0.011 - 0.134	0.746 0.622 - 0.841	0.179 0.099 - 0.295	0.029 0.004 - 0.112	0.449 (67)
AOM	0.034 0.002 - 0.195	0.586 0.391 - 0.759	0.103 0.027 - 0.284	0.276 0.134 - 0.475	0.195 (29)
Perf	0.00	0.385 0.152 - 0.678	0.154 0.102 - 0.224	0.461 0.204 - 0.738	0.087 (13)
Total	0.107 (16)	0.604 (90)	0.174 (26)	0.114 (17)	1.00 (149)

Numbers in parentheses are numbers of examinations. Abbreviations as on Table 3.

TABLE 5. TABLE OF CONDITIONAL PROBABILITIES OF TRANSITIONS, AND THEIR CONFIDENCE INTERVALS, BETWEEN EAR STATES FOR INDIVIDUAL NON-ABORIGINAL EARS EXAMINED AT INTERVALS OF 15 TO 30 DAYS

	15 to 30 Days Later			
Earlier	*Normal*	*OME*	*AOM*	*Total*
Normal	0.785	0.169	0.046	0.833
	0.662 - 0.874	0.091 - 0.287	0.012 - 0.137	(65)
OME	1.0	0.00	0.00	0.115
	0.629 - 0.989			(9)
AOM	0.25	0.00	0.75	0.051
	0.013 - 0.78		0.219 - 0.987	(4)
Total	0.782	0.141	0.077	1.00
	(61)	(11)	(6)	(78)

Numbers in parentheses are numbers of examinations. Abbreviations as on Table 3.

original schoolchildren's binaural health may already be established in the first months of infancy in some Aboriginal children. Further study needs to examine this possibility; our observations ceased at child age 12 months, while McCafferty et al reported 9 years of observation starting with children 6 years old, and more observation, especially between 12 months and 5 years, is required to confirm this possibility. Alternatively, a cross-sectional study of Aboriginal children between 1 year of age and school age would clarify the possible persistence of the binaural patterns.

If the early separation of children into distinct binaural health patterns can be confirmed, and underlying reasons elucidated, this information would be valuable for the early detection and management of Aboriginal infants with OM and conductive hearing loss to minimize developmental consequences. By implication, failure to provide effective treatment in the first year may condemn affected children to one of the long-term patterns described by McCafferty et al[6] and confirmed here. It is known, for instance, that children with perforations are likely to suffer more severe hearing loss,[16] and so the children of highest priority are those whose binaural patterns suggest long-term TM perforation and who may benefit most from early detection and treatment. Even so, all early OM should be treated to avoid potential long-term otologic, developmental, and educational problems.

ACKNOWLEDGMENTS — The authors thank the families enrolled in the study for their continuing involvement, and staff in the Royal Darwin Hospital postnatal ward and at the island's community health center for their cooperation and patience. Thanks also to Gill Campbell, Sue Keagan, and Kathy Kemp for assistance with data collection, to John Vorrath for otoscopy training, to research assistants Colleen Kantilla and Mondullulu Koops, and to Jenny Powers for statistical advice.

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TWENTIETH ANNUAL MEETING OF THE BÁRÁNY SOCIETY

The Twentieth Annual Meeting of the Bárány Society will be held in Würzburg, Germany, September 20-23, 1998. For more information, contact the Local Organizing Committee, Institute of the 4-G-F, Kurhausstrasse 12, D 97688 Bad Kissingen, Germany; telephone 0049/971-64832; fax 0049/ 971-68637.

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NEUROMONITORING IN OTOLARYNGOLOGY AND SKULL BASE SURGERY

Neuromonitoring in Otolaryngology and Skull Base Surgery will be held in London, England, February 21, 1997. For information, contact Dianne Rooksby, Centre for Continuing Professional Development, University of Nottingham, University Park, Nottingham, NG7 2RD, United Kingdom; telephone 44 115 951 3763; fax 44 115 951 3722; Email dianne.rooksby@nottingham.ac.uk.

Ann Otol Rhinol Laryngol 105:1996

DIAGNOSING NASAL HYPERREACTIVITY WITH POSITIONAL RHINOMANOMETRY

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Positional rhinomanometry is a physiologic method for estimating nasal resistance, which is variable, difficult to predict, and linked to vasomotor activity of the functional system culminating in the turbinate valves. Thirty subjects suffering from allergic rhinitis, 25 patients affected by aspecific rhinitis, and 40 healthy controls underwent positional rhinomanometry. Test positions included the seated (baseline), supine, and recumbent (homolateral and contralateral to the nasal fossa under examination). In patients with perennial allergic rhinitis and in those with aspecific rhinitis, positional rhinomanometry elicited two pathologic responses: either an average percentage rise of more than 80% in nasal resistance in the supine and homolateral and contralateral recumbent positions compared with basal values, or a paradoxical fall in the supine and homolateral recumbent positions.

KEY WORDS — nasal hyperreactivity, positional rhinomanometry.

INTRODUCTION

Homeostatic regulation of the microcirculation in nasal mucosa is extremely complex. Adrenergic vasoconstriction[1] and cholinergic vasodilation[2] interact, and the activity of neuromediators such as vasoactive intestinal polypeptide, substance P, neuropeptide Y, and neurochinine A must also be taken into consideration.[3] All contribute to the complex functioning of the vasomotor system that culminates in the turbinate valves. Wang and Jackson[4] hypothesized presynaptic parasympathetic control of sympathetic nerve fibers. Neurovegetative dystonia associated with laxity of the turbinal cavernous tissue plays, in vasomotor rhinitis, an important role in the genesis of symptoms such as nasal obstruction that is particularly marked when reclining. Indeed, the effect of changes in position on turbinal tissue is a physiologic method for measuring nasal resistance, which tends to vary and is difficult to predict. The standing position has long been known (since Kaiser's[5] observations in 1895) as a physiologic condition that modifies nasal patency. Increased resistance in the supine position appears to be linked to a rise in blood pressure in the internal jugular vein and the consequent congestion of turbinal cavernous tissue.[6-8] However, turbinal congestion when reclining does not depend only on hydrostatics. The neurovegetative system specifically influences filling capacitance vessels, and this effect is particularly marked in the lateral recumbent position. Homolateral nasal fossae congestion on reclining and contralateral decongestion are caused by the reflex disactivation of sympathetic tone due to the pressure exerted on intercostal receptors on one side of the body.[9-12] Compressing the underarm cavity produces the same effect.[13]

Rhinomanometry in different positions (positional rhinomanometry) has often been used in recent years to evaluate nasal resistance.[14-18]

This study attempts to provide a quantitative assessment of changes in nasal resistance induced by the recumbent positions in both specific and aspecific nasal hyperreactivity.

MATERIALS AND METHODS

Anterior active positional rhinomanometry was performed with a digital rhinomanometer (Amplifon Markos NR4) in 55 patients affected by nasal hyperreactivity and in 40 healthy controls.

In conformity with European standards,[19] airflow expressed in cubic centimeters per second was measured at a preselected pressure value of 150 Pa. Resistance levels (the mean of four consecutive respirations) were calculated from Poiseuille's law ($R = \Delta p/V$). All tests were carried out at standard temperature (22°C to 26°C) and humidity (50% to 60%) levels. No test lasted more than 30 to 35 minutes, in order to avoid nasal cycle interference. After a 30-minute acclimatization period with the seated patient breathing spontaneously through a face mask, recordings for each nasal fossa were taken as follows.

1. Basal values with the patient seated.
2. Supine values (head versus trunk at a 20° angle);

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TABLE 1. PERCENTAGE VARIATIONS IN NASAL RESISTANCE

	Normal Subjects (n = 40)	*Group A (Specific Nasal Hyper-reactivity; n = 30)*	*Group B (Aspecific Nasal Hyper-reactivity; n = 25)*
Supine	+29% ± 39%	+16% ± 100%	+65% ± 137%
Homolateral	+59% ± 87%	+118% ± 185%	+133% ± 249%
Contralateral	+17% ± 58%	+82% ± 302%	+51% ± 212%

Data are mean ± SD.

this position significantly increases pressure in the internal jugular vein.[6]

3. Recumbent values homolateral to the nasal fossa being examined.
4. Recumbent values contralateral to the nasal fossa being examined.

Each recording was made immediately after the patient had rested for 3 minutes in the new position. Percentage variations in nasal resistance compared with basal values (seated) were calculated in order to avoid the scatter found in absolute values that is due to great interindividual and intraindividual differences.

The 55 patients were divided into two groups on the basis of the specificity or aspecificity of nasal hyperreactivity.

Group A included 30 patients (11 women, 19 men, average age 30.8 years) affected by specific nasal hyperreactivity, which was diagnosed on the basis of 1) case histories, 2) clinical signs (sneezing, rhinorrhea, seasonal or perennial nasal obstruction), 3) objective rhinologic examination (patients with concomitant organic abnormalities in the vestibulum and nasal septum were excluded from the study), 4) nasal provocation test, and 5) skin and/or radioimmunologic tests.

These patients were divided into two subgroups on the basis of whether hyperreactivity was seasonal or perennial.

Eighteen (60%) of the 30 patients were considered to be affected by seasonal allergic rhinitis; the other 12 (40%) by the perennial form. Allergometric tests showed those with seasonal rhinitis reacted prevalently to Graminaceae, and the others to different allergens, but mainly to *Dermatophagoides*. Positional rhinomanometry tests were performed in the noncritical period when patients were not under therapy.

Group B included 25 patients (15 women, 10 men, average age 27.4 years) affected by aspecific nasal hyperreactivity. Inclusion criteria were the nonseasonal clinical picture, persistent symptoms of nasal obstruction (which was markedly more severe in the recumbent position), and negative provocation nasal, skin, and/or radioimmunologic allergy tests.

TABLE 2. CASES OF INCREASED AND DECREASED NASAL RESISTANCE

	Increased Resistance		*Decreased Resistance*	
	No.	*%*	*No.*	*%*
Group A (seasonal allergic rhinitis; 18 patients, 36 nasal fossae)				
Supine	18	50	18	50
Homolateral	26	72.2	10	27.8
Contralateral	22	61.1	14	38.9
Group A (perennial allergic rhinitis; 12 patients, 24 nasal fossae)				
Supine	14	58.3	10	41.7
Homolateral	14	58.3	10	41.7
Contralateral	15	62.5	9	37.5
Group B (aspecific vasomotor rhinitis; 25 patients, 50 nasal fossae)				
Supine	29	57	21	43
Homolateral	31	62	19	38
Contralateral	24	47	26	53

Positional rhinomanometry testing was carried out in the less severe periods when patients had suspended therapy. Student's *t*-test for unpaired data was used for the statistical analysis of results; p values less than .05 were considered significant and are reported here.

RESULTS

Table 1 reports the general trend of the results of positional rhinomanometry in the 30 patients (60 nasal fossae) in group A, in the 25 (50 nasal fossae) in group B, and in the 40 healthy controls. Results are expressed as percentage variations in resistance compared to baseline (seated) values. Table 2 separates increases and decreases in nasal resistance in the two subgroups in group A (specific seasonal and perennial rhinitis) and in group B (aspecific rhinitis). Only percentage increases in resistance were compared with those of the control group in Table 3. Compared with controls, significant differences in nasal resistance were observed in patients with perennial rhinitis and in those with aspecific rhinitis (Table 3).

DISCUSSION

The general trend in rhinomanometric responses should be read in the light of both increases and

TABLE 3. PERCENTAGE INCREASES IN NASAL RESISTANCE

	Normal Subjects (n = 40)	*Group A (Seasonal Allergic Rhinitis; n = 18)*	*Group A (Perennial Allergic Rhinitis; n = 12)*	*Group B (Aspecific Vasomotor Rhinitis; n = 25)*
Supine	+35% ± 28% (92%)	+64% ± 57% (50%)	+72% ± 48%* (58.3%)	+138% ± 209%* (57%)
Homolateral	+61% ± 49% (89%)	+115% ± 73% (72.2%)	+167% ± 64%* (58.3%)	+213% ± 297%* (62%)
Contralateral	+23% ± 18% (75%)	+111% ± 108% (61.1%)	+120% ± 30%* (62.5%)	+157% ± 359%* (47%)

Data are mean ± SD. Percentages in parentheses refer to nasal fossae.

*$p < .005$ versus normal controls in corresponding positions.

decreases in nasal resistance in all positions in each of the two groups. Alterations that exist in healthy subjects[16] are more marked in patients with nasal hyperreactivity (whether specific or aspecific), as the high scatter in the values of the standard deviation indicates, suggesting that turbinal cavernous tissue "disreactivity" is caused by changes in position.

Interindividual nasal resistance is known to vary greatly in the recumbent position. Within-subject variability is a potential source of error in any study on nasal resistance.[20,21] Our study was designed to reduce this variation as much as possible. The nasal pressure tube was not removed at each change of position, thus reducing the margin of error caused by face mask placement and replacement, especially in lateral recumbency. All measurements were carried out in as little time as possible in order to avoid dynamic changes in nasal resistance caused by spending a larger period of time in the decubitus position.[20] This is also the reason for the main limitation of this study design, in that responses cannot be considered partial, maximal, or complete, but only as indicative of "disreactivity."

In healthy subjects nasal resistance tends to increase in the supine and lateral recumbent positions[16] (Table 1), while in many of the patients, independently of whether hyperreactivity was specific or not, values of nasal resistance dropped in these positions (Table 2). Reduced resistance is physiologic in normal subjects only in the contralateral recumbent position, because of decongestion of the upper nasal fossae.[12]

However, rhinomanometry indicated a slight increase in resistance in the contralateral recumbent position[16] in 75% of the nasal fossae in normal subjects, and a drop in resistance in the other 25% (Table 3). In other words, the slight increase is probably due to the physiologic decongestion of the upper nasal fossae[9-12] associated with the collapse of the valve walls.[22]

The decrease in resistance in the supine and homolateral recumbent positions that we observed in both groups of patients is a strangely paradoxical response (Table 2). It is probably due to the activation of the sympathetic nerve tone, rather than the disactivation that is to be expected in the homolateral recumbent position. The reason for this abnormal response is difficult to understand, and further studies aimed specifically in this direction are undoubtedly necessary. Whatever the cause, this paradoxical response is often considered evidence of vasomotor rhinitis, whether specific or aspecific.[14]

Differential analysis of increases in resistance in group A (with the subgroups of seasonal and perennial rhinitis) and group B shows different patterns of changes in nasal resistance.

Compared with healthy subjects, nasal resistance increased more in group A and was associated with a smaller standard deviation in patients with perennial rhinitis, while in those with seasonal rhinitis, average increases were smaller and associated with a greater scatter in the standard deviation. Therefore, rhinomanometric data on perennial allergic rhinitis indicate more compact and less dispersive values of nasal resistance compared with seasonal allergic rhinitis. Differences in the increased resistance in the two subgroups and the healthy controls were significant in all positions only in the group with perennial rhinitis (Table 3). As we know, this enhancement of the functional turbinate valve response is associated with abnormal cavernous tissue laxity in the recumbent position. Percentage increases in nasal resistance in group B were higher in all positions and standard deviation scatter was greater compared with healthy subjects. These data indicate a turbinal cavernous tissue abnormal response that was quantitatively more marked than in perennial allergic rhinitis and that was confirmed by the high values of the statistical significance (Table 3).

CONCLUSION

Positional rhinomanometry revealed a disreactivity in specific and aspecific rhinitis manifested in two phenomena, the first being the paradoxical response in the supine and homolateral recumbent positions, and the second a percentage increase in nasal resistance of more than 80% in all positions. In any positional rhinomanometry test, either one or both abnormalities are markers of vasomotor rhinitis.

ACKNOWLEDGMENT — The authors thank Dr G. A. Boyd for the translation of this paper.

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Ann Otol Rhinol Laryngol 105:1996

BRAIN STEM AUDITORY-EVOKED POTENTIALS OF MICE WITH EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS

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Experimental allergic encephalomyelitis (EAE) is an experimental model for multiple sclerosis. In order to study autoimmune retrocochlear hearing loss, we performed brain stem auditory-evoked potential (BAEP) studies on EAE mice. The EAE was induced in B10.PL and (PL/J × SJL)F1 mice. In the BAEP study, all of the peak and interpeak latencies were prolonged significantly in the diseased mice. Hearing thresholds were slightly elevated in the immunized mice during the acute phase. Inflammatory and phagocytic cell infiltration, demyelination, and Vβ8.1, 8.2 T-cell receptor–positive cells were observed in the cochlear nerves or their proximity by histologic study. It is suggested that immunologic reactions identified with EAE also occurred in the cochlear nerve, and that these reactions were responsible for the hearing problems. It appears that EAE is a useful model system for studying autoimmune insults on the neural portion of the auditory system.

KEY WORDS — demyelination, evoked potentials, experimental allergic encephalomyelitis, mouse, multiple sclerosis.

INTRODUCTION

Multiple sclerosis (MS) is an autoimmune disease pathologically characterized by perivascular invasion of the central nervous system (CNS) by mononuclear lymphocytes with consequent inflammation and demyelination of the neuron sheaths; this leads to neurologic deficits up to and including paralysis and death. The auditory system is unquestionably affected by MS. The percentages of abnormal brain stem auditory-evoked potentials (BAEPs) in MS patients reported in the literature range between 0% and 93%. The average is almost 60%.[1] It has been thought that this wide spectrum of percentages can be due to difficult criteria either in MS staging or in BAEP evaluation.[2] The BAEPs in MS patients are characterized by prolonged latency, an increased I-V interval, a diminished amplitude of wave V, and morphologically degraded, poorly reproducible waveforms.[3]

Experimental allergic encephalomyelitis (EAE) is considered to be an animal model of MS.[4-8] In the murine model, mice develop a demyelinating disease characterized by uncontrolled shivering, neuromuscular deficit, and coordination difficulties.[9] These are similar to the clinical and pathologic changes observed in MS in humans. The immunologic mechanisms underlying the disease process have been well characterized. Like MS, EAE is a major histocompatibility complex (MHC) class II–linked disease, with H-2^u and H-2^s being the most susceptible haplotypes.[9] Two of the highest responding strains are B10.PL and (PL/J × SJL/J)F1.[9] Encephalitogenic T cells from B10.PL mice respond to a myelin basic protein (MBP) N-terminal peptide (NAc1-9) in association with the I-A^u MHC molecule.[10,11] (PL/J × SJL/J)F1 mice potentially express both I-A^u and I-A^s molecules. However, insufficient expression of I-A^s was observed in (PL/J × SJL/J)F1 mice.[12,13] Therefore, encephalitogenic T cells recognize the same N-terminal epitope as that of B10.PL mice in (PL/J × SJL/J)F1 mice. More than 80% of encephalitogenic T lymphocytes from H-2^u background mice are expressing the T cell antigen receptor (TCR) Vβ8.2 gene segment.[11] The encephalitogenic T cells from (PL/J × SJL/J)F1 mice also predominantly express the Vβ8.2 gene segment.[10] Despite these similarities in the T cell responses to antigen, the course of the disease is markedly different. Under the conditions used in this study, (PL/J × SJL/J)F1 mice develop a moderate degree of paralysis that usually follows a relapse-remission pattern. The B10.PL mice develop a more severe paralysis, most often resulting in the death of the animal.

We had studied the auditory function in shiverer

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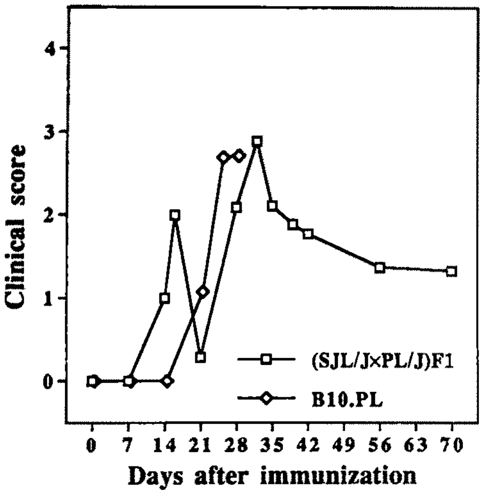

Fig 1. Clinical score of mice with experimental allergic encephalomyelitis (EAE). Value given is average score.

mice with BAEPs.[14] These mice showed clear hearing conduction delays. The shiverer mice are an autosomal recessive mutation of the MBP structural gene, and are characterized by generalized tremors, seizures, and a shortened life span. The mutation affects primarily the CNS, in which the absence of MBP results in poorly compacted myelin in the oligodendrocyte sheath. The lesions in EAE resemble those found in the shiverer mice, except that the autoimmune lesions are patchy as opposed to systemic; the EAE lesions tend to be locally severe and can vary with time in a relapse-remission pattern.

Auditory involvement in MS is not infrequent. The proximal portion of the cochlear nerve is sheathed by MBP-expressing oligodendrocytes, and is therefore a potential target for the demyelination induced by immunization with MBP in EAE. It is of interest to know if the mouse EAE model exhibits auditory lesions similar to those of human MS. If this proved to be the case, this mouse model could prove valuable for evaluation of potential treatment modalities. This led us to examine the hearing of EAE mice. Both B10.PL and (PL/J × SJL/J)F1 mice were used in this study to examine the differences that might arise because of variance in the genetic background. Both male and female mice were used to check for possible sex differences.

TABLE1. NUMBER OF MICE TESTED FOR HEARING

Days After Immunization	*(PL/J × SJL/J)F1*		*B10.PL*	
	EAE	*Control*	*EAE*	*Control*
0	14	15	13	13
7	10	11	13	13
14	9	11	13	12
16	8	11		
21	7	11	13	12
25			13	6
28	10	11	7	11
32	9	11		
35	9	11		
39	9	10		
42	9	11		
56	8	7		
70	9	11		

EAE — experimental allergic encephalomyelitis.

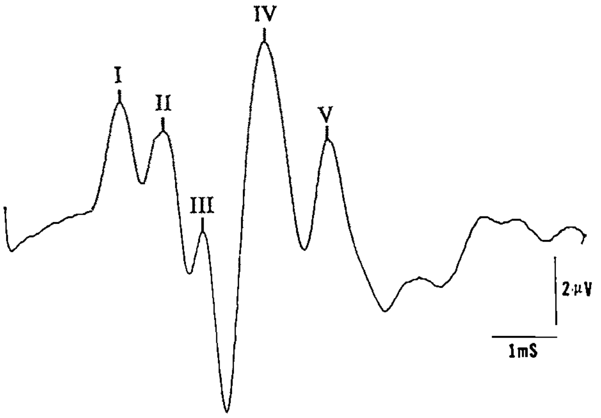

Fig 2. Typical brain stem auditory-evoked potential (BAEP) tracing indicating numbering of peaks. Indicated are points used to measure peak and interpeak latencies.

MATERIALS AND METHODS

Animals. B10.PL and (PL/J × SJL/J)F1 mice were used in this study. Both sexes of B10.PL mice, male PL/J mice, and female SJL/J mice were obtained from The Jackson Laboratories (Bar Harbor, Me) and bred at the Memphis Veterans Administration Medical Center Animal Research Facility. Animals were from 6 to 8 weeks old at the time of disease induction.

Antigen. The MBP was prepared from frozen rat brain (Pel-Freez Biological, Rogers, Ark) by the method of Smith.[15] Briefly, the dissected rat brains were homogenized in 0.32 mol/L sucrose and layered onto 0.85 mol/L sucrose. After the centrifugation (25,000 rpm for 30 minutes), the interface was carefully removed, washed three times with deionized water, and lyophilized overnight. The protein was resuspended in 90% acetone and stirred for 30 minutes at room temperature. The precipitate was dried and redissolved in 10 mL 0.3N hydrochloric acid. The supernatant was carefully removed and dialyzed against 10 mmol/L acetic acid. The acetic acid was removed by lyophilization, and the concentration of MBP was deduced by 12% sodium dodecyl sulfate gel electrophoresis.

Induction of EAE. The EAE was induced by a modification of a standard protocol.[11] The mice were given a subcutaneous injection of 200 μg of MBP and killed *Mycobacterium tuberculosis* (H37RA, Difco, Detroit, Mich) at a concentration of 2.5 mg/mL in a

TABLE 2. PEAK LATENCIES

		Peak Latencies (milliseconds; mean ± SD)				
Days	*Group*	*I*	*II*	*III*	*IV*	*V*
(PL/J × SJL/J)F1 mice						
0	MBP	1.96 ± 0.03	2.73 ± 0.06	3.41 ± 0.09	4.54 ± 0.10	5.69 ± 0.18
	Control	1.95 ± 0.03	2.70 ± 0.03	3.42 ± 0.05	4.51 ± 0.07	5.61 ± 0.12
7	MBP	1.91 ± 0.03	2.74 ± 0.07	3.43 ± 0.05	4.48 ± 0.09	5.60 ± 0.13
	Control	1.92 ± 0.05	2.68 ± 0.06	3.40 ± 0.11	4.45 ± 0.11	5.53 ± 0.15
14	MBP	1.97 ± 0.06*	2.88 ± 0.17†	3.71 ± 0.32*	4.81 ± 0.36†	5.93 ± 0.38†
	Control	1.92 ± 0.06	2.71 ± 0.05	3.48 ± 0.07	4.47 ± 0.08	5.55 ± 0.12
16	MBP	2.06 ± 0.10†	3.03 ± 0.23†	3.80 ± 0.31†	5.05 ± 0.50†	6.22 ± 0.58†
	Control	1.92 ± 0.03	2.71 ± 0.05	3.45 ± 0.10	4.44 ± 0.11	5.52 ± 0.16
21	MBP	2.04 ± 0.08†	2.90 ± 0.17†	3.61 ± 0.12†	4.65 ± 0.24†	5.91 ± 0.33†
	Control	1.94 ± 0.03	2.70 ± 0.05	3.44 ± 0.06	4.40 ± 0.05	5.52 ± 0.11
28	MBP	2.05 ± 0.12†	2.95 ± 0.18†	3.72 ± 0.24†	4.89 ± 0.37†	6.12 ± 0.51†
	Control	1.93 ± 0.04	2.69 ± 0.08	3.39 ± 0.09	4.37 ± 0.07	5.47 ± 0.09
32	MBP	2.04 ± 0.13	2.92 ± 0.17†	3.67 ± 0.22†	4.77 ± 0.29†	5.96 ± 0.33†
	Control	1.96 ± 0.04	2.71 ± 0.05	3.42 ± 0.07	4.39 ± 0.10	5.50 ± 0.11
35	MBP	1.97 ± 0.05	2.83 ± 0.15†	3.61 ± 0.13†	4.57 ± 0.18†	5.67 ± 0.30
	Control	1.95 ± 0.02	2.68 ± 0.03	3.39 ± 0.06	4.38 ± 0.08	5.48 ± 0.13
39	MBP	1.98 ± 0.05	2.86 ± 0.16*	3.62 ± 0.20†	4.63 ± 0.32	5.84 ± 0.37*
	Control	1.97 ± 0.06	2.70 ± 0.07	3.39 ± 0.11	4.39 ± 0.18	5.50 ± 0.23
42	MBP	1.98 ± 0.13	2.80 ± 0.12*	3.60 ± 0.19†	4.58 ± 0.22*	5.73 ± 0.23
	Control	1.96 ± 0.04	2.71 ± 0.06	3.40 ± 0.06	4.41 ± 0.10	5.56 ± 0.19
56	MBP	1.96 ± 0.09	2.77 ± 0.15	3.47 ± 0.20	4.52 ± 0.20*	5.61 ± 0.31
	Control	1.96 ± 0.04	2.68 ± 0.05	3.33 ± 0.06	4.29 ± 0.13	5.40 ± 0.11
70	MBP	1.92 ± 0.13	2.78 ± 0.19	3.52 ± 0.16	4.51 ± 0.29	5.68 ± 0.32
	Control	1.95 ± 0.03	2.75 ± 0.03	3.41 ± 0.09	4.40 ± 0.07	5.52 ± 0.10
B10.PL mice						
0	MBP	2.09 ± 0.04	2.85 ± 0.06	3.48 ± 0.08	4.61 ± 0.16	5.67 ± 0.16
	Control	2.09 ± 0.03	2.86 ± 0.04	3.47 ± 0.07	4.64 ± 0.09	5.61 ± 0.09
7	MBP	2.11 ± 0.05	2.84 ± 0.07	3.45 ± 0.08	4.61 ± 0.12	5.64 ± 0.15
	Control	2.08 ± 0.03	2.86 ± 0.04	3.48 ± 0.06	4.59 ± 0.11	5.56 ± 0.15
14	MBP	2.13 ± 0.05	2.90 ± 0.07	3.52 ± 0.11	4.69 ± 0.14	5.71 ± 0.17
	Control	2.10 ± 0.04	2.87 ± 0.05	3.45 ± 0.08	4.62 ± 0.13	5.63 ± 0.13
21	MBP	2.17 ± 0.08	3.01 ± 0.20	3.63 ± 0.25	4.80 ± 0.31	5.96 ± 0.50
	Control	2.13 ± 0.05	2.90 ± 0.07	3.51 ± 0.11	4.67 ± 0.14	5.68 ± 0.20
25	MBP	2.30 ± 0.17†	3.31 ± 0.35†	4.08 ± 0.51*	5.37 ± 0.71*	6.78 ± 1.00*
	Control	2.07 ± 0.09	2.85 ± 0.13	3.47 ± 0.10	4.53 ± 0.16	5.62 ± 0.26
28	MBP	2.19 ± 0.11	3.08 ± 0.19†	3.75 ± 0.19*	5.02 ± 0.35†	6.14 ± 0.31†
	Control	2.11 ± 0.06	2.88 ± 0.09	3.55 ± 0.11	4.66 ± 0.16	5.68 ± 0.04

Days — days after immunization, MBP — myelin basic protein.
*$p < .05$ compared with control.
†$p < .01$ compared with control.

0.1-mL emulsion consisting of equal volumes of 10 mmol/L acetic acid and complete Freund's adjuvant. Three hundred nanograms of *Bordetella pertussis* toxin (List Pharmaceuticals, Campbell, Calif) were given intravenously 24 and 72 hours later. Note that subcutaneous injection of antigen, rather than the standard foot pad injection, was used to minimize discomfort to the experimental animals. The quality of commercial *B pertussis* preparations is highly variable and the dose used was worked out in advance experiments. Control animals were inoculated with an emulsion of complete Freund's adjuvant, phosphate-buffered saline (PBS), and *M tuberculosis*, accompanied by intravenous injection of 300 ng of pertussis toxin 24 and 72 hours later.

All animals were followed for clinical signs of disease and BAEPs until they were painlessly sacrificed for tissue or until resolution of their disease. The following scale was utilized to grade the clinical status of the mice: 0, no clinical disease; 1, tail weakness; 2, paraparesis; 3, paraplegia; and 4, quad-

TABLE 3. INTERPEAK LATENCIES

		Interpeak Latencies (milliseconds; mean ± SD)			
Days	*Group*	*I-II*	*I-III*	*I-IV*	*I-V*
(PL/J × SJL/J)F1 mice					
0	MBP	0.77 ± 0.05	1.45 ± 0.07	2.58 ± 0.10	3.73 ± 0.16
	Control	0.76 ± 0.03	1.45 ± 0.05	2.57 ± 0.07	3.67 ± 0.12
7	MBP	0.82 ± 0.08	1.52 ± 0.06	2.56 ± 0.10	3.69 ± 0.13
	Control	0.76 ± 0.06	1.48 ± 0.09	2.53 ± 0.11	3.61 ± 0.13
14	MBP	0.91 ± 0.14*	1.74 ± 0.30	2.84 ± 0.33*	3.96 ± 0.34†
	Control	0.79 ± 0.05	1.56 ± 0.08	2.55 ± 0.07	3.63 ± 0.11
16	MBP	0.86 ± 0.10†	1.57 ± 0.05†	2.61 ± 0.18†	3.87 ± 0.25†
	Control	0.78 ± 0.03	1.53 ± 0.08	2.51 ± 0.08	3.60 ± 0.14
21	MBP	0.85 ± 0.09*	1.56 ± 0.05*	2.62 ± 0.16*	3.84 ± 0.23†
	Control	0.76 ± 0.05	1.50 ± 0.06	2.46 ± 0.05	3.58 ± 0.11
28	MBP	0.93 ± 0.11†	1.68 ± 0.16†	2.84 ± 0.30†	4.07 ± 0.43†
	Control	0.76 ± 0.06	1.46 ± 0.09	2.44 ± 0.06	3.55 ± 0.07
32	MBP	0.91 ± 0.12†	1.63 ± 0.12†	2.72 ± 0.23†	3.92 ± 0.28†
	Control	0.75 ± 0.03	1.46 ± 0.05	2.43 ± 0.10	3.53 ± 0.10
35	MBP	0.86 ± 0.12†	1.64 ± 0.14†	2.60 ± 0.17†	3.70 ± 0.30
	Control	0.73 ± 0.03	1.44 ± 0.05	2.43 ± 0.07	3.53 ± 0.13
39	MBP	0.88 ± 0.14†	1.64 ± 0.19†	2.65 ± 0.30*	3.87 ± 0.33*
	Control	0.73 ± 0.04	1.42 ± 0.11	2.42 ± 0.15	3.53 ± 0.18
42	MBP	0.83 ± 0.15*	1.62 ± 0.15†	2.60 ± 0.13†	3.75 ± 0.16
	Control	0.75 ± 0.05	1.44 ± 0.06	2.45 ± 0.09	3.60 ± 0.17
56	MBP	0.81 ± 0.14	1.51 ± 0.18	2.56 ± 0.15*	3.65 ± 0.24*
	Control	0.72 ± 0.02	1.37 ± 0.05	2.33 ± 0.10	3.44 ± 0.08
70	MBP	0.86 ± 0.12	1.61 ± 0.11†	2.59 ± 0.19*	3.76 ± 0.22†
	Control	0.80 ± 0.03	1.46 ± 0.10	2.46 ± 0.06	3.57 ± 0.08
B10.PL mice					
0	MBP	0.76 ± 0.06	1.39 ± 0.07	2.52 ± 0.15	3.59 ± 0.13
	Control	0.77 ± 0.04	1.38 ± 0.07	2.56 ± 0.08	3.52 ± 0.08
7	MBP	0.73 ± 0.07	1.35 ± 0.05*	2.50 ± 0.10	3.53 ± 0.11
	Control	0.77 ± 0.04	1.39 ± 0.05	2.51 ± 0.10	3.48 ± 0.14
14	MBP	0.77 ± 0.05	1.39 ± 0.08	2.56 ± 0.11	3.59 ± 0.14
	Control	0.77 ± 0.04	1.35 ± 0.08	2.52 ± 0.12	3.53 ± 0.12
21	MBP	0.84 ± 0.14	1.45 ± 0.18	2.63 ± 0.25	3.79 ± 0.42
	Control	0.77 ± 0.04	1.38 ± 0.10	2.54 ± 0.11	3.55 ± 0.16
25	MBP	1.01 ± 0.20*	1.78 ± 0.39*	3.07 ± 0.58*	4.48 ± 0.86*
	Control	0.78 ± 0.08	1.40 ± 0.06	2.46 ± 0.10	3.55 ± 0.19
28	MBP	0.89 ± 0.11†	1.56 ± 0.09*	2.83 ± 0.27†	3.95 ± 0.21†
	Control	0.77 ± 0.06	1.44 ± 0.09	2.54 ± 0.12	3.57 ± 0.18

Days — days after immunization, MBP — myelin basic protein.

*p < .05 compared with control.

†p < .01 compared with control.

riplegia or moribundity. This modifies a standard five-point scale[16] for evaluating the status of mice by combining quadriplegia with moribundity, since in our animal facility, quadriplegic mice are euthanized.

Recording of BAEPs. The BAEPs were recorded by a Nicolet CA-1000 system (Nicolet Biomedical Instruments, Madison, Wis) with an intraperitoneal injection of anesthetic (a mixture of 85 mg/kg ketamine hydrochloride and 17 mg/kg xylazine). One hundred–microsecond clicks were generated at a repetition rate of 11.4 per second as the sound stimulations and then delivered by an earphone to an external ear canal of the mouse through a 21-cm connecting tube. The evoked potentials, taken with needle electrodes inserted subcutaneously at the vertex and ipsilateral paw, were amplified $\times 10^4$, filtered with a band pass between 150 and 1,500 Hz, and averaged from a total of 500 click-evoked responses for the first 10-millisecond period following stimulation. The minimum detectable value on the two different points of the latency, measured by the cursor mode of this system,

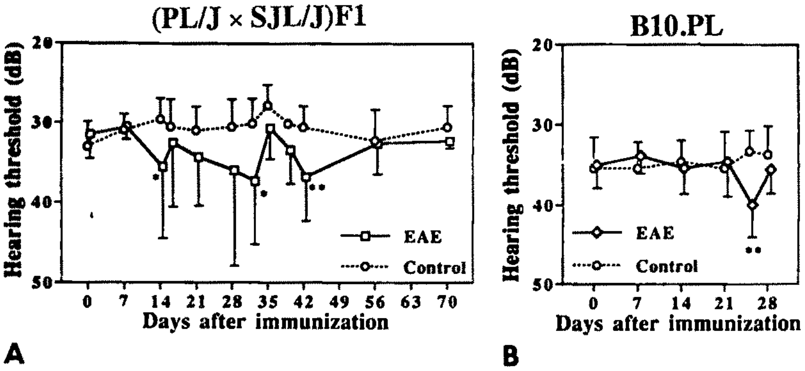

Fig 3. Comparison of hearing thresholds in decibels hearing level of A) (PL/J × SJL/J)F1 and B) B10.PL mice. * —$p<.05$. ** —$p<.01$. Values are average threshold ± SD.

is 0.04 millisecond. The minimal hearing thresholds were determined with 5-dB–step sound stimulation according to the visually detectable amplitude of the first peak. On the basis of studies of mice from our previous report,[14] we measured peak and interpeak latencies by a 70-dB sound stimulation 5 minutes after anesthetic injection. Hearing thresholds are in decibels hearing level (HL) and are based on measurements in over 50 control mice in preliminary work.

Histologic Studies. For light microscopy, mice were anesthetized, painlessly sacrificed, and dissected. The head was immersed in 10% buffered formaldehyde solution. Tissues were decalcified with 10% ethylenediaminetetraacetic acid, dehydrated, and embedded in paraffin. Sections of 6 μm cut vertically and horizontally were stained with hematoxylin and eosin.

For immunohistochemistry, the brain and temporal bone tissues were fixed by transfusion via the heart with periodate-lysine-paraformaldehyde solution. The dissected tissues were immersed in the solution for 24 hours at 4°C. The specimen was rinsed with 0.01 mol/L of PBS (pH 7.4) containing 10% to 20% sucrose for 36 hours at 4°C, embedded in OCT (optimal cutting temperature) compound (Miles Laboratories Inc, Elkhart, Ind), and immediately frozen. Frozen sections of 6 μm in thickness were obtained. After rehydrating and rinsing in cold PBS, the endogenous pseudoperoxidase was blocked with absolute methanol containing 0.5% hydrogen peroxide for 20 minutes at room temperature, and then the sections were treated with 3% normal goat serum in PBS to reduce the nonspecific reaction. Biotin-conjugated mouse anti mouse Vβ8.1, 8.2 TCR monoclonal antibodies (Pharmingen, San Diego, Calif), diluted to 1:50 in PBS containing 0.5% bovine serum albumin, were applied to the sections and incubated 24 hours at 4°C. After rinsing with PBS, the sections were incubated with avidin-biotin horseradish peroxidase complexes (Vectastain Elite ABC Kit, Vector Laboratories, Inc, Burlingame, Calif) for 30 minutes at room temperature and rinsed sufficiently with PBS. The reaction product was developed with 0.02% 3,3′-diaminobenzidine in 0.05 mol/L of Tris buffer (pH 7.6) with 0.005% hydrogen peroxide for 7 minutes. The sections, with counterstaining, were dehydrated, cleared in xylene, and mounted. Control staining for specificity of antibodies was studied with PBS instead of antibodies.

TABLE 4. SUMMARY OF MORPHOLOGIC FINDINGS

Group		*Acute Phase*	*70 dPI*
(PL/J × SJL/J)F1	EAE	++:1, +:3, ±:1	++:2, −:1
	Control	±:1, −:1	−:3
B10.PL	EAE	++:2, +:4	0
	Control	±:1	0

Symbols −, ±, +, and ++ refer to degree of cell infiltration observed in cochlear nerve. Number after colon in each case refers to number of mice with given level of infiltration.

dPI — days postimmunization, EAE — experimental allergic encephalomyelitis (immunized with myelin basic protein).

For transmission electron microscopy, mice were anesthetized, painlessly sacrificed by transfusion via the heart with Karnovsky's fixative (2.5% glutaraldehyde and 2% formaldehyde in 0.2 mol/L of cacodylate buffer; pH 7.4), and dissected to immerse the head in the same fixative at 4°C until use. The cochlear nerves were taken from the specimen macroscopically. These specimens were postfixed in buffered 2% osmium tetroxide, dehydrated through graded concentrations of ethanol, and embedded in epoxy resin (Epon). Semithin sections were stained with toluidine blue and observed by light microscope. Thin sections were stained with uranyl acetate and lead citrate. Observations were made with a transmission electron microscope (JEM-1200EX, Jeol, Tokyo, Japan).

Statistical Analyses. Statistical analyses were performed by Student's *t*-test or the χ^2 test, as appropriate.

RESULTS

Clinical Observations. We immunized 16 (PL/J × SJL/J)F1 mice (6 males and 10 females) and 13 B10.PL mice (9 males and 4 females) with MBP. The

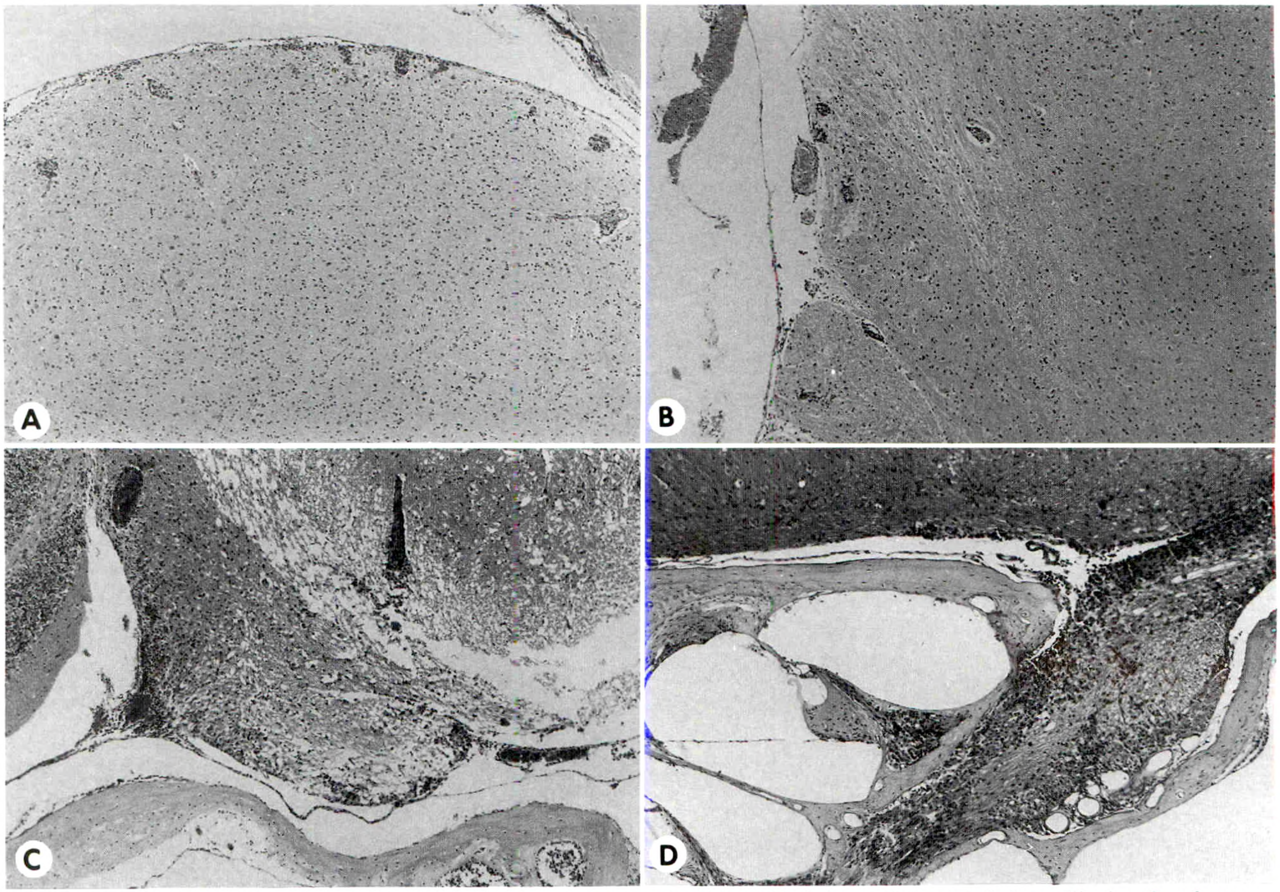

Fig 4. Inflammatory cell infiltration in auditory tract (paraffin section, H & E, original ×100). A) Inferior colliculus. Specimen was obtained from (PL/J × SJL/J)F1 mouse at 16 days postimmunization (dPI). At this time, clinical grade was 2. B) Lateral lemniscus. This specimen comes from same mouse as in A. C) Cochlear nucleus. This specimen was obtained from B10.PL mouse at 25 dPI. At this time, clinical grade was 2 and hearing threshold was 40 dB HL. D) Cochlear nerve. This specimen was obtained from B10.PL mouse at 25 dPI. At this time, clinical grade was 4 and hearing threshold was 45 dB HL.

numbers of the control mice were 15 in (PL/J × SJL/J)F1 mice (5 males and 10 females) and 13 in B10.PL mice (9 males and 4 females). The incidences of clinical signs in the immunized mice were 67% in male (PL/J × SJL/J)F1 mice and 100% in the other groups of immunized mice. The onset of the clinical signs was 20 ± 6.4 (mean ± SD) days postimmunization (dPI) in male (PL/J × SJL/J)F1 mice, 17 ± 3.5 dPI in male (PL/J × SJL/J)F1 mice, 22 ± 2.4 dPI in male B10.PL mice, and 25 ± 1.7 dPI in female B10.PL mice. There were no significant differences between males and females regarding the incidence and onset of clinical signs. In (PL/J × SJL/J)F1 mice, the clinical signs were improved from 21 to 28 dPI and the mice were showing signs of relapse at 28 dPI (Fig 1). Five out of 9 of these mice still had clinical signs (grade 2 or 3) at 70 dPI. Most of the B10.PL mice were dead after 28 dPI.

Comparison of Peak Latencies. Analysis of hearing tests was performed by using the data from mice that presented clinical signs. Table 1 shows the numbers of mice on which we performed hearing tests. The first through fifth peaks were reliably obtained from most of the mice (Fig 2). Some peaks were not identified in 1 B10.PL mouse and 4 (PL/J × SJL/J)F1 mice immunized with MBP; the details are as follows: peaks III and IV at 35 dPI in a B10.PL mouse, peaks II to V at 28 dPI and peak II at 30 dPI in a (PL/J × SJL/J)F1 mouse, peak II at 28 to 32 dPI in a (PL/J × SJL/J)F1 mouse, and peak III at 70 dPI in 2 (PL/J × SJL/J)F1 mice.

In (PL/J × SJL/J)F1 mice immunized with MBP, the delay of peak latencies was observed from 14 dPI. The delay increased at 16 dPI, once decreased at 21 dPI, and increased, thereafter, to a maximum at 28 dPI. After 28 dPI, the delay was decreasing. At 70 dPI there was no significant difference between the immunized and control groups, although half of these mice still had clinical signs of EAE (Table 2). In B10.PL mice, the peak was delayed significantly from 25 dPI. Further observation was impossible, because most of the mice were dead after 28 dPI (Table 2). Compared with the average of the clinical score, the delay of the peak seemed to be parallel to

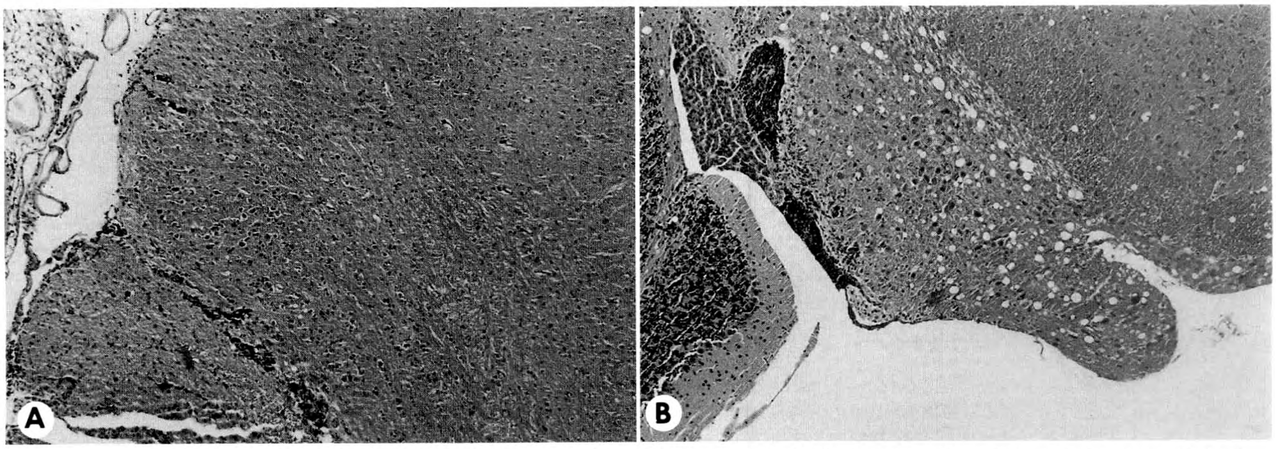

Fig 5. Inflammatory cell infiltrations at 70 dPI (paraffin section, H & E, original ×100). A) Lateral lemniscus. At this time, clinical grade was 3 and hearing threshold was 30 dB HL. B) Cochlear nucleus. At this time, clinical grade was 0 and hearing threshold was 35 dB HL.

the severity of clinical signs on the whole (Fig 1 and Table 2). At 21 dPI, when the clinical signs were improved, the delay of the peak was also getting smaller in (PL/J × SJL/J)F1 mice immunized with MBP.

Comparison of Interpeak Latencies. The interpeak latencies were also prolonged at the same time as the delay of peak latencies. The prolongations were maximum at 28 dPI in (PL/J × SJL/J)F1 mice, and were maximum at 25 and 28 dPI in B10.PL mice. These interpeak prolongations were decreasing with time in the same way as the delay of peak latencies. However, significant prolongation remained at 70 dPI in the (PL/J × SJL/J)F1 mice. Significant prolongation was observed in interpeaks I-III, I-IV, and I-V with MBP immunization (Table 3).

Comparison of Minimal Hearing Thresholds. In (PL/J × SJL/J)F1 mice, the averaged minimal hearing thresholds were elevated significantly compared with controls at 14, 32, and 42 dPI with MBP. In B10.PL mice, the averaged minimal hearing thresholds were elevated significantly at 25 dPI. The elevation was from 5 to 10 dB HL (Fig 3). The hearing threshold was elevated to 70 dB HL at 28 dPI in 1 (PL/J × SJL/J)F1 mouse immunized with MBP.

Histologic Findings. We observed 6 immunized mice (from 23 to 39 dPI) and a control mouse (35 dPI) among B10.PL mice, and 5 immunized mice (from 16 to 32 dPI) and 2 control mice (14 and 21 dPI) among (PL/J × SJL/J)F1 mice in paraffin sections under a light microscope. The results are summarized in Table 4. In the acute phase, mononuclear inflammatory cell infiltrations were observed in the spiral ganglion, cochlear nerve, cochlear nucleus, superior olive, lateral lemniscus, and inferior colliculus (Fig 4) by light microscopic observation. There were no inflammatory changes in the cochlea. Three immunized and 3 control (PL/J × SJL/J)F1 mice were observed at 70 dPI under a light microscope (Table 4). The cell infiltration was still observed in the

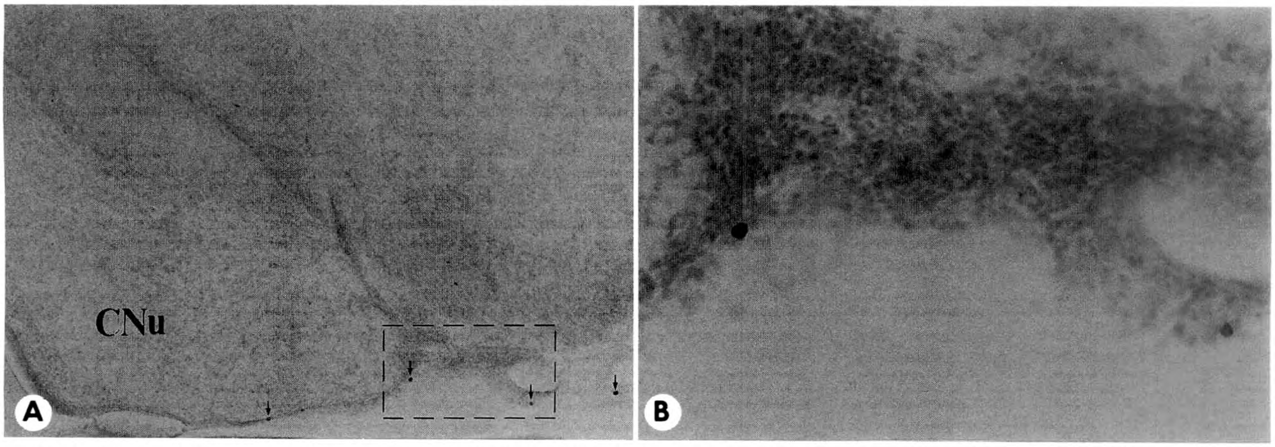

Fig 6. Immunohistochemical demonstration of TCR Vβ8.1, 8.2–positive cells in vicinity of cochlear nucleus. This specimen comes from B10.PL mouse at 28 dPI. At this time, clinical grade was 2 and hearing threshold was 30 dB HL. A) Original ×100. CNu — cochlear nucleus, arrows — positive cells. B) Higher magnification of boxed area in A (original ×400).

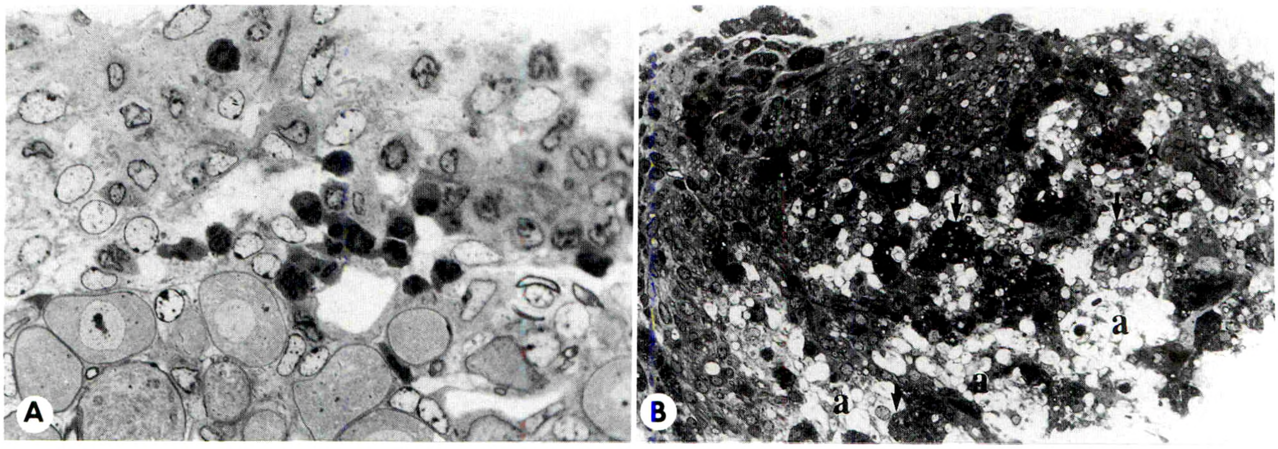

Fig 7. Light micrographs taken from Epon sections of cochlear nerve in acute EAE. A) Cochlear nerve from B10.PL mouse at 25 dPI (original ×1,000). Hearing threshold was 45 dB HL and clinical grade was 4. Polymorphonuclear lymphocytes had infiltrated into area adjacent to vestibular ganglion. B) Cochlear nerve from (PL/J × SJL/J)F1 mouse at 30 dPI (original ×400). Hearing threshold was 30 dB HL and clinical grade was 2. Hearing threshold had been elevated to 70 dB HL at 28 dPI. Macrophage-containing myelin debris (arrows) and naked axons (a) were seen.

cochlear nucleus and lateral lemniscus of (PL/J × SJL/J)F1 mice (Fig 5). In the cochlear nerve, we were not able to find cell infiltration. There were no severe cell infiltrations in control mice.

Immunohistochemical studies were performed on 3 immunized (28, 28, and 32 dPI) and 2 control (28 and 32 dPI) B10.PL mice, and 1 (30 dPI) (PL/J × SJL/J)F1 mouse. We found Vβ8.1, 8.2 TCR–positive cells in the subependymal area close to the inferior colliculus, lateral lemniscus, and cochlear nucleus (Fig 6). In control mice, we were not able to observe TCR-positive cells in the auditory tract.

Three immunized (25, 25, and 28 dPI) B10.PL mice, a control (28 dPI) B10.PL mouse, and an immunized (30 dPI) (PL/J × SJL/J)F1 mouse were observed by transmission electron microscopy. We found polymorphonuclear lymphocyte infiltration in the vestibular ganglion, macrophages, and demyelination in Epon semithin sections from immunized mice but not the control mouse (Fig 7). In transmission electron microscopy, naked axons, myelin debris in extracellular spaces (Fig 8A), and macrophages containing membranous material in cytoplasmic vacuoles (Fig 8B) were observed. We also examined 2 immunized mice and a control (PL/J × SJL/J)F1 mouse at 70 dPI. Demyelinated areas still could be observed in the cochlear nerve (Fig 9). Inflammatory and phagocytic cell infiltrations were not significant. Significant changes were not observed in control mice by transmission electron microscopy.

DISCUSSION

The human auditory system is unquestionably

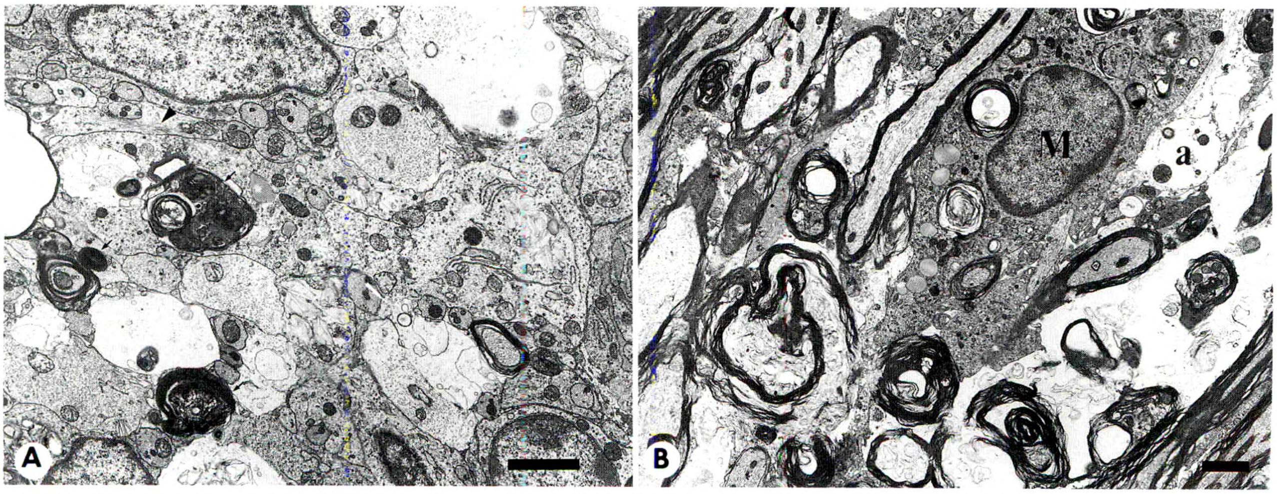

Fig 8. Electron micrographs of cochlear nerve from immunized B10.PL mouse. Hearing threshold was 45 dB HL and clinical grade was 4 at 25 dPI. Bars — 2 μm. A) Naked axon and myelin debris (arrows) were seen. Astrocytic gliosis was seen also (arrowhead). B) Macrophage (M) and phagocytosed myelin sheath. In cytoplasm, phagocytosed myelin debris was seen in vesicle. Naked axons (a) were seen around macrophage.

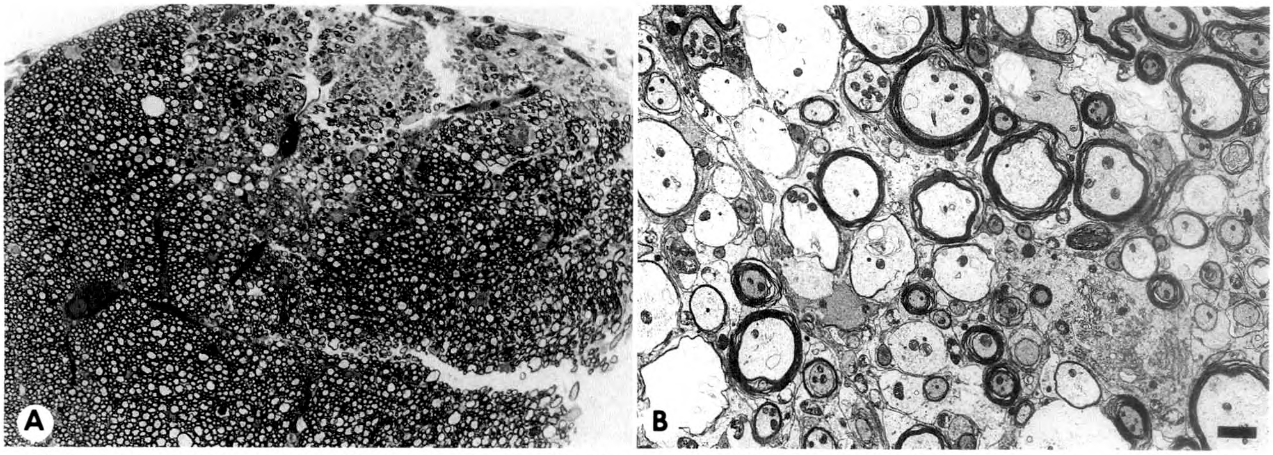

Fig 9. A) Light micrograph taken from Epon sections of cochlear nerve at 70 dPI. Demyelinated fibers were still scattered (arrow). This (PL/J × SJL/J)F1 mouse showed no clinical signs and had 30 dB HL hearing threshold (original ×400). B) Electron micrograph from A. Naked axons and thinly myelinated axons are evident on transmission electron microscopy. Bar — 2 μm.

affected by MS; the average of reported percentages of abnormal BAEPs in MS patients is almost 60%.[1] In MS patients, BAEPs are characterized by prolonged latency, an increased I-V interval, a diminished amplitude of wave V, and morphologically degraded, poorly reproducible waveforms.[3] In this study, we have investigated the auditory consequences of MBP-induced EAE in mice; this experimental disease is thought to be a good model of human MS. In our mouse EAE model, the mice that displayed clinical signs of EAE also showed significant prolongation of peak latencies and interpeak latencies. This prolongation was observed in all peaks and interpeaks. The differences between the immunized and control groups were more clear in the central part of the auditory tract (peaks IV and V and interpeaks I-IV and I-V) than in the peripheral part (peaks I and II and interpeak I-II), in concordance with the human disease. However, the delay of peak I is consistent with the inflammatory cell infiltration in the cochlear nerve that we observed. There are several case reports mentioning prolongation of wave I latency in MS patients.[17-20] In contrast to the human MS studies, we were not able to frequently observe a diminished amplitude of wave V or morphologically degraded, poorly reproducible waveforms. The reason for this difference between humans and mice is not yet clear. Our study focused on the acute phase of EAE. Some of the (PL/J × SJL/J)F1 mice had clinical signs at 70 dPI. The delays of peak and interpeak latencies were improved at that time. In most of the human studies, the patients had a history of relapsing and remitting illness. Unfortunately, we were not able to observe the relapsing of clinical signs in this study; we need long-term observation as a next step. Our study did clearly show the delay of the peak and interpeak latencies over the period of observation, so we believe that this animal model is valid for the study of auditory involvement in demyelinating diseases such as MS.

TABLE 5. THRESHOLD EFFECT ON PEAK AND INTERPEAK LATENCIES FOR CONTROL B10.PL MICE

	40 dB HL Mice (N = 15)	*35 dB HL Mice (N = 23)*
Peaks		
I	2.072 ± 0.041	2.080 ± 0.051
II	2.843 ± 0.049	2.831 ± 0.054
III	3.499 ± 0.092	3.463 ± 0.054
IV	4.584 ± 0.135	4.553 ± 0.085
V	5.680 ± 0.199	5.614 ± 0.145
Interpeaks		
I-II	0.771 ± 0.053	0.751 ± 0.040
I-III	1.427 ± 0.090	1.383 ± 0.065
I-IV	2.512 ± 0.121	2.473 ± 0.016
I-V	3.608 ± 0.186	3.534 ± 0.125

Latencies are mean ± SD in milliseconds.

It might be argued that the abnormalities of the BAEPs result from decreases in the hearing threshold due to peripheral deficits. This appears to be unlikely for a number of reasons. First, the difference in average thresholds between MBP-immunized mice and control mice was small, generally 5 dB HL or less at most time points. A comparison of peak and interpeak latencies between 15 control B10.PL mice with thresholds of 40 dB HL and 23 matched control B10.PL mice with thresholds of 35 dB HL showed no statistical differences (Table 5). In contrast, the MBP-immunized mice had statistically significant changes in peak and interpeak latencies, even at times when there were no statistical differences in hearing thresholds. Furthermore, the greatest changes in latencies occurred in the peak and interpeak (peaks IV and V and interpeaks I-IV and I-V) latencies that represent the central portion of the auditory response; statistically significant changes were observed in these latencies, even at times when peak I latencies were normal. Therefore, we believe these results are truly representative of retrocochlear hearing loss.

There is no fundamental agreement in the clinical literature regarding the elevated thresholds of pure tone audiometry that are produced by MS.[1,21] However, there is agreement regarding sudden sensorineural hearing loss in MS patients. This has been reported as an uncommon presenting symptom in this disease.[1] There was a slight elevation of mean thresholds in our mouse EAE model. The inflammatory cell infiltration in the cochlear nerve that was observed could account for this. One out of 29 mice immunized with MBP developed a considerable elevation of hearing threshold, which seems analogous to the human sudden sensorineural hearing loss in MS, although there is insufficient evidence to demonstrate strict equivalence.

Our histologic findings coincided with the results of BAEPs. Four out of 9 immunized (PL/J × SJL/J)F1 mice still had paraparesis or paraplegia at 70 dPI. We found inflammatory cell infiltrations in the cochlear nucleus and lateral lemniscus, but not in the cochlear nerve. Peak I latencies were returning to normal, but interpeak latencies were still prolonged slightly. However, we still found demyelinated areas in the cochlear nerve. The same situation was reported in the CNS of EAE mice.[22] The number of remyelinated axons was small even in mice that had multiple relapses and remissions. Onofrj et al[23] reported that latency reductions are found in guinea pigs in which remyelination phenomena are not present. It is well known that the extent of lesions in MS is in many cases far greater than would be anticipated from clinical observations. Asymptomatic or "silent" demyelinated MS lesions have been described in the spinal cord.[24] Furthermore, the prolongation of interpeak latencies was not so great as that of the acute phase. Therefore, there may be some compensative reaction after acute inflammation. For example, Moll et al[25] reported the altered distribution of voltage-sensitive sodium channels in human MS patients.

The elevation of hearing thresholds, prolongation of peak I latency, and pathologic changes in the cochlear nerve seen by light microscopy indicated that the disorder known to occur in the CNS might extend to the cochlear nerve. Because of this, we focused on the cochlear nerve in our transmission electron microscopy. The pathologic changes in the earliest stage of acute EAE are small lymphocytes and polymorphonuclear cell infiltration in submeningeal and perivascular areas, hemorrhagic lesions, a loss of myelinated axons by wallerian degeneration, and appearance of small numbers of naked axons. The CNS lesions showed a decrease in the number of inflammatory cells, an increase in the proportion of phagocytic cells in the infiltrates, and an increase in the number of naked axons. As animals begin to improve clinically from the acute attack, minimal remyelination and astrocytic gliosis become pronounced.[22] Our transmission electron microscopic observation showed almost the same findings in the cochlear nerve.

Immunologically, EAE has been used as an animal model to study T cell–mediated autoimmune diseases in humans. More than 80% of encephalitogenic T lymphocytes from B10.PL or (PL/J × SJL/J)F1 mice express the TCR Vβ8.2 gene segment.[10,11] Although we were not able to show Vβ8 TCR–positive cells in the cochlear nerve itself, Vβ8 TCR–positive cells were shown in the vicinity of the cochlear nucleus and nerve by our immunohistochemical study.

Putting these findings together, we found a strong suggestion that the audiologic changes of EAE mice were comparable to those of MS patients, and that immunologic reactions identified with EAE in the cochlear nerve and CNS were responsible for the audiologic abnormalities. It appears that EAE mice are a suitable model for investigating immune insults to the auditory tract of MS patients. To our knowledge, there is only one previous report regarding hearing tests in an EAE model (Onofrj et al[23]), which used guinea pigs as an experimental animal. More precise dissection of immunologic events during autoimmune involvement may be possible with our mouse model, since a great deal more is known about the genetics and epigenetic phenomena affecting the immune system in the mouse than is known in the guinea pig model. The eventual goal of this ongoing study is to develop a method of ameliorating or abrogating the autoimmune destruction of the CNS and restoring audiologic and other function. We believe that EAE mice are a useful model for studying autoimmune insults to the neural portion of the auditory system.

ACKNOWLEDGMENTS — T. Watanabe expresses his gratitude to Goro Mogi, MD, Professor and Chairman, the Department of Otolaryngology, Medical University of Oita, Japan, for giving him an opportunity to study abroad in Professor Yoo's laboratory. The authors thank Loretta Hatmaker of the Electron Microscopy Laboratory at the Veterans Administration Medical Center, Memphis, for her excellent technical assistance.

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Ann Otol Rhinol Laryngol 105:1996

EFFECT OF LIPOXYGENASE INHIBITION ON MUCOUS GLYCOPROTEIN SECRETION FROM CHINCHILLA MIDDLE EAR EPITHELIAL CELLS IN VITRO

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Lipoxygenase is an enzyme that metabolizes arachidonic acid down to leukotrienes. Recent studies have shown that the enzyme is implicated in mucous glycoprotein (MGP) secretion stimulated by inflammatory mediators in the airways, suggesting its possible role in secretion of MGP from middle ear epithelial cells. To investigate a correlation between MGP secretion and the arachidonic acid metabolites, we examined the effects of nordihydroguaretic acid (NDGA, both a cyclooxygenase and lipoxygenase inhibitor), low-dose indomethacin (an inhibitor of cyclooxygenase), and A63162 (an inhibitor of lipoxygenase) on MGP secretion in cultured chinchilla middle ear epithelial cells. It was found that lipoxygenase inhibition led to reduction of MGP secretion from cultured chinchilla middle ear epithelial cells, while cyclooxygenase inhibition did not. Both cyclooxygenase and lipoxygenase inhibition resulted in profound blockage of MGP secretion in baseline and platelet activating factor–stimulated MGP secretion. It was concluded, therefore, that MGP secretion was linked to arachidonic acid metabolites, especially lipoxygenase products.

KEY WORDS — lipoxygenase, middle ear epithelial cells in vitro, mucous glycoprotein secretion, otitis media with effusion, platelet activating factor.

INTRODUCTION

Platelet activating factor (PAF) is a low–molecular weight, ether-linked phospholipid released by a variety of inflammatory cell types, including basophils, platelets, mast cells, eosinophils, neutrophils, monocytes, and macrophages, as well as by endothelium, epithelium, and perhaps other, as yet unidentified cells.[1-4] Platelet activating factor is a potent bioactive mediator that is implicated in inflammatory, allergic, and immune reactions. It has been shown that PAF is a potent secretagogue of mucous glycoprotein (MGP) and plays an important role in the pathogenesis of otitis media with effusion in vivo and in vitro.[5,6] Because of its ability to stimulate MGP release, PAF appears to be an important mediator that causes hypersecretion of mucus in the middle ear cavity and eustachian tube. This action appears to be mediated by PAF receptors on epithelial cell surfaces.[7] However, the mechanism of mucous secretion at the postreceptor level is not understood.

Arachidonic acid metabolism is known to play an important role in otitis media. Prostaglandin and leukotriene levels increased in child and adult patients with otitis media.[8,9] Recent studies have suggested that arachidonic acid metabolites are involved in MGP secretion in the airways. Work by Adler et al[10] demonstrated an increase in leukotrienes in the supernatant of cells incubated with the inflammatory mediator PAF. Further studies indicated that exogenous 5-, 12-, and 15-hydroxyeicosatetraenoic acids stimulated MGP secretion from cultured guinea pig respiratory epithelium,[11] implying that lipoxygenase products may trigger MGP secretion.

Mucus hypersecretion, caused by inflammatory mediators, could be a serious problem in patients with otitis media, because of the increase of viscoelasticity of the middle ear fluid. It is believed that increased viscoelasticity of middle ear effusion is, in part, responsible for disturbances of the mucociliary transport system of the middle ear. It is important, therefore, in the treatment of this disease, to inhibit excessive secretion from middle ear epithelial cells. In the present study, a possible link between the arachidonic acid metabolites and MGP secretion was examined by means of cultured chinchilla middle ear epithelial cells. To determine which pathway is significant to MGP secretion, we selectively blocked the lipoxygenase pathway and/or cyclooxygenase path-

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From the Otitis Media Research Center, Departments of Otolaryngology (Lin, Lees, Ondrey, Juhn) and Pediatrics (Kim), University of Minnesota School of Medicine, Minneapolis, Minnesota. Supported by grant P01-DC00133 from the National Institutes of Health/National Institute on Deafness and Other Communication Disorders. This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Minnesota.

Presented at the meeting of the American Academy of Otolaryngology–Head and Neck Surgery, Minneapolis, Minnesota, October 2-6, 1993.

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way.

MATERIALS AND METHODS

Reagents. Nordihydroguaretic acid (NDGA), indomethacin, streptomycin sulfate, amphotericin B, chondroitinase ABC, putrescine, dithiothreitol, sodium azide, insulin-transferrin–sodium selenite (ITS), and PAF were purchased from Sigma Chemical Company, St Louis, Mo. The PAF was dissolved with chloroform. In all experiments described below, chloroform was chosen as the appropriate control vehicle (with the same amount used as in the experimental groups). Ecoscint A was purchased from National Diagnostic Laboratory, Atlanta, Ga; ^{3}H-glucosamine from NEN, Boston, Mass; Dulbecco's modified Eagle's medium mixture/F-12 (DMEM/F-12) and fetal bovine serum (FBS) from Gibco BRL, Grand Island, NY; and A63162 from Abbott, Chicago, Ill.

Primary Cell Culture. Healthy young chinchillas weighing 400 to 600 g were used as donors of middle ear epithelial cells. Prior to the experiment, the chinchillas' tympanic membranes were examined under an operating microscope to confirm that the middle ears were free of infection. Chinchillas were anesthetized with ketamine hydrochloride (20 mg/kg) and euthanized by decapitation, and both tympanic bullae were removed. The eustachian tubes were ligated, and the bullae were injected with 0.09% protease in DMEM-F12 with 0.5% FBS, 1% ITS, hydrocortisone (50 ng/mL), penicillin G sulfate (100 µg/mL), streptomycin sulfate (100 µg/mL), and amphotericin B (250 ng/mL), wrapped with Parafilm, and incubated at 4°C for approximately 16 hours. The suspension containing the middle ear epithelial cells was aspirated and the bullae were rinsed several times with a washing solution (the above media containing 10% FBS, without protease). Suspensions were pooled and centrifuged at 500*g* for 10 minutes, and the pellet was washed again with fresh washing solution. These procedures were repeated three times to completely remove the protease. The pellet containing the middle ear epithelial cells was resuspended in 1 mL of full growth culture medium (DMEM/F-12) containing 0.5% FBS, ITS (insulin 5 µg/mL, transferrin 5 µg/mL, sodium selenite 5 ng/mL), hydrocortisone (50 ng/mL), penicillin G sulfate (100 U/mL), streptomycin sulfate (100 µg/mL), and amphotericin B (250 ng/mL). The total cell number per 1 mL was determined with a hemocytometer. Approximately 5×10^4 cells (in 1.5 mL culture media) were seeded on each Celagen membrane disc and placed in a humidified atmosphere containing 95% air–5% carbon dioxide. Putrescine (5 mmol/L) was added to the cultures between days 2 and 3 to suppress fibroblast proliferation. When the middle ear epithelial cells reached subconfluency, the cells were ready for experimentation.

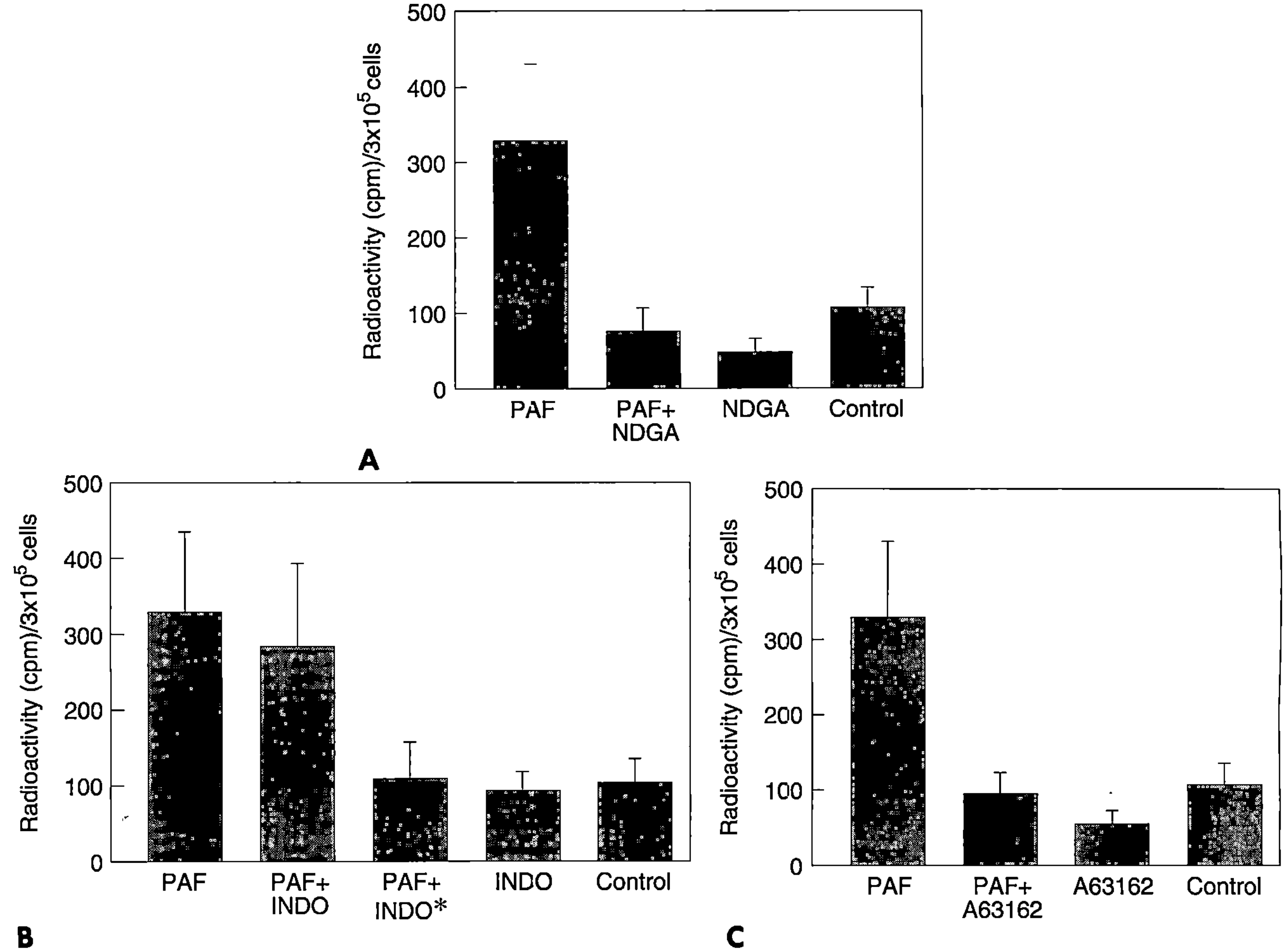
Metabolic Labeling of MGP. Cultures were metabolically labeled by incubation with 1.5 mL per well of full growth culture medium containing 5 µCi/mL tritiated glucosamine for 24 hours after the epithelial cells reached subconfluency.

Quantitation of MGP. The MGP was assayed according to our previous work,[12,13] in which high–molecular weight glycoconjugates excluded from a Sepharose CL-4B column and resistant to proteoglycan-digesting enzymes were identified as MGP. Supernatant samples were treated with 0.4 U/mL of testicular chondroitinase ABC at 37°C for 5 hours to remove proteoglycans. After incubation, the digestion mixture was neutralized to pH 7.4 with 0.1 mol/L acetic acid and applied to the Sepharose CL-4B column equilibrated with phosphate-buffered saline (PBS) containing 0.02% (wt/vol) sodium azide. Columns were eluted with PBS containing 0.02% sodium azide, 0.9% sodium chloride, and 5 mmol/L dithiothreitol at a constant flow rate of 0.5 mL/min, and fractions of 2 mL were collected. Void volume fractions were mixed with 1 mL of Ecoscint A, and the radioactivity of fractions was counted with a liquid scintillation spectrometer. Radioactivity at peak fractions was defined as the amount of MGP in the supernatant. The radioactivity from each sample was divided by its own viable cell number, and the individual value of radioactivity, on average, was calculated. The radioactivity of every 3×10^5 cells of each group is presented in the Figure.

Lipoxygenase and Cyclooxygenase Inhibition of Cells. Cultures were radiolabeled with 5 µCi of ^{3}H-glucosamine for 24 hours and divided into experimental groups: control versus NDGA; and PAF versus PAF + NDGA.

To evaluate the effect of cyclooxygenase and lipoxygenase inhibition on baseline MGP secretion, the cultures were preincubated with and without NDGA (a mixed cyclooxygenase and lipoxygenase inhibitor) at a concentration of 1 mmol/L for 24 hours, and then incubated with and without NDGA at the same concentration for 4 hours. Supernatants were collected for determination of MGP levels. To evaluate the effect of cyclooxygenase and lipoxygenase inhibition on PAF-stimulated MGP secretion, the cultures were preincubated with and without NDGA at a concentration of 1 mmol/L for 24 hours, and then incubated with PAF and PAF + NDGA for 4 hours. Supernatants were collected for determination of MGP levels.

To determine which pathway was significant for MGP secretion, inhibition of either the cyclooxy-



Results. PAF — platelet activating factor, control — full growth medium plus PAF vehicle. A) Effect of nordihydroguaretic acid (NDGA) on baseline and PAF-stimulated mucous glycoprotein (MGP) secretion. NDGA, at concentration of 1 mmol/L, exhibited inhibitory effect on MGP secretion in baseline and PAF-stimulated MGP states (NDGA versus control, $p < .05$; PAF + NDGA versus PAF, $p < .05$). This suggests that MGP secretion in cultured middle ear epithelial cells may be related to arachidonic acid metabolites. PAF concentration was 100 µmol/L. Radioactivity values are means ± SE for $n = 6$ at each point. B) Effect of indomethacin on baseline and PAF-stimulated MGP secretion. Indomethacin, at concentration of 2.5 µmol/L (low dose, inhibition of cyclooxygenase), did not significantly inhibit PAF-stimulated MGP secretion (INDO versus control, $p > .05$; PAF + INDO versus PAF, $p > .05$). However, indomethacin at concentration of 10 µmol/L (high dose, inhibition of both cyclooxygenase and lipoxygenase) inhibited baseline and PAF-stimulated MGP secretion (PAF + INDO* versus PAF, $p < .05$). This suggests that baseline and PAF-stimulated MGP secretion in cultured middle ear epithelial cells acts through lipoxygenase pathway. Radioactivity values are means ± SE for $n = 6$ at each point. C) Effect of A63162 on baseline and PAF-stimulated MGP secretion. A63162, at concentration of 20 µmol/L, significantly inhibited baseline and PAF-stimulated MGP secretion (A63162 versus control, $p < .05$; PAF + A63162 versus PAF, $p < .05$). This indicates that baseline and PAF-stimulated MGP secretion is mediated by lipoxygenase pathway. Radioactivity values are means ± SE for $n = 6$ at each point.

genase pathway or the lipoxygenase pathway was carried out. Experimental groups were as follows: control versus INDO (indomethacin, low concentration); PAF versus PAF + INDO; PAF versus PAF + INDO* (indomethacin, high concentration); control versus A63162; and PAF versus PAF + A63162. To evaluate the effect of cyclooxygenase inhibition on baseline MGP secretion, the cultures were labeled as mentioned above and preincubated with and without indomethacin at 2.5 µmol/L (low concentration, inhibiting cyclooxygenase only) for 24 hours, and then incubated with and without indomethacin at the same concentration for 4 hours. Supernatants were harvested for determination of MGP levels. To evaluate the effect of cyclooxygenase inhibition on PAF-stimulated MGP secretion, the cultures were preincubated with and without indomethacin at 2.5 µmol/L and 10 µmol/L (high concentration, inhibiting both cyclooxygenase and lipoxygenase) for 24 hours, and then incubated with and without indomethacin at the same concentrations for 4 hours. Supernatants were harvested for determination of MGP levels. To evaluate the effect of lipoxygenase inhibition on baseline and PAF-stimulated MGP secretion, in a similar way, the cultures were preincubated with and without A63162 (a specific lipoxygenase inhibitor) for 24 hours, and then incubated with and without A63162 for 4 hours (in the baseline state) or incubated with PAF and PAF + A63162 (in the PAF-stimulated state). Supernatants were then harvested for determination of MGP levels. Cells were collected after trypsin–ethylenediaminetetraacetic acid treatment to examine cell number and viability. After determination of total cell numbers, the average secretory activity for every 3×10^5 cells was calculated.

Statistics. Differences between groups were assessed by Student's *t*-test for unpaired samples, with $p < .05$ considered statistically significant. In the experiments to analyze the effects of NDGA, indomethacin, and A63162 on MGP secretion in the baseline and PAF-stimulated states, six replicate tissue culture plates were used to generate each experimental point. The results are expressed as standardized radioactivity of 3×10^5 cells and are provided as the mean ± SE.

RESULTS

Morphologically, the primary culture of chinchilla middle ear epithelial cells at subconfluency was a monolayer consisting of polygonal cells, as reported previously.[14] The culture contained few ciliated cells in the early days (days 1 through 4). No ciliated cells were identified after day 7. Few fibroblasts (<5%) were observed.

Inhibition of Both Cyclooxygenase and Lipoxygenase Pathways. Platelet activating factor, at a concentration of 100 μmol/L, significantly stimulated MGP secretion from cultured chinchilla middle ear epithelial cells, as reported previously.[14] At 1 mmol/L, NDGA significantly decreased PAF-induced secretion of MGP (PAF versus PAF + NDGA, $p < .05$). It restored the secretion of MGP to the control levels, and significantly decreased the baseline secretion of MGP from the cells (see Figure, A). This indicates that arachidonic acid metabolites play an important physiologic and pathologic role in MGP secretion. There was no difference between medium alone and medium plus the PAF vehicle, as reported previously.[6]

Inhibition of Cyclooxygenase Pathway. Indomethacin, at a concentration of 2.5 μmol/L, which inhibits the cyclooxygenase pathway only, did not block the PAF-stimulated secretions (PAF versus PAF + INDO, $p > .05$, see Figure, B) or the baseline secretions (control versus INDO, $p > .05$, see Figure, B); this finding indicates that the autocrine level of intracellular prostaglandins does not affect MGP secretion. However, at a concentration of 10 μmol/L, indomethacin significantly decreased PAF-stimulated MGP secretion (PAF versus PAF + INDO*, $p < .05$). Low-dose indomethacin inhibited the cyclooxygenase pathway only, while high-dose indomethacin inhibited both the lipoxygenase and cyclooxygenase pathways. This suggests that baseline and PAF-stimulated MGP secretion in cultured middle ear epithelial cells acts through the lipoxygenase pathway.

Inhibition of Lipoxygenase Pathway. At a concentration of 20 μmol/L, A63162 significantly reduced MGP secretion (see Figure, C). The A63162, which inhibits the lipoxygenase pathway only, demonstrated an inhibitory effect on both PAF-induced MGP secretion (PAF versus A63162, $p < .05$) and baseline secretion (control versus A63162, $p < .05$). This further confirms that lipoxygenase metabolites are important in MGP secretion under normal and pathologic conditions.

Viability of Cells. A consistent rate of cell viability was seen with trypan blue staining of the control groups (control and PAF-treated groups), ranging from 75% to 88%. The rates were from 73% to 89% in the experimental groups (NDGA, INDO, and A63162 groups). There were no differences between controls and PAF, NDGA, INDO, and A63162 groups, or between the PAF and PAF + NDGA, PAF + INDO, PAF + INDO*, and PAF + A63162 groups. No apparent cytotoxic effects were observed.

DISCUSSION

There are two ways in which PAF-stimulated MGP secretion can be inhibited. One is to block PAF from binding to its receptor on the target cell surface (receptor level). The other is to block signal transduction at the postreceptor level or biochemical pathway.

Platelet activating factor is known to induce responses in target cells by binding to specific receptors.[7,15-18] To block the PAF-stimulated effect on target cells, antagonists of the PAF receptor such as WEB 2170 BS could be used, as reported previously.[7] However, all the antagonists studied exhibited incomplete inhibition, although some showed promising results in animal models. Each antagonist studied so far was less efficient in binding to the receptor than PAF. For example, the concentrations of receptor antagonists required to inhibit specific binding of PAF to its receptor on polymorphonuclear leukocytes by 50% (ED50) were between 30- and 1,000-fold higher than the concentration of PAF. Some antagonists have agonist activity at high concentrations due to their similarity in structure to PAF. Those structural analogs also competitively inhibit PAF hydrolysis by plasma PAF acetylhydrolase, although this may not significantly alter PAF half-life in vivo.[19] These disadvantages limit application of PAF receptor antagonists in human studies, and other approaches to reduction of MGP secretion should be studied.

Binding of PAF to its receptor is known to initiate a variety of intracellular signaling transductions and biochemical pathways.[20-23] There has been evidence that PAF-stimulated cells release free arachidonic acid and metabolize it to eicosanoids.[14,24] Mucous glycoprotein secretion was associated with lipoxygenase metabolite elevation in the airways, and the exogenous leukotriene enhanced secretion of MGP. Studies have confirmed that inhibition of the lipoxygenase pathway reduces MGP secretion, leading to the assumption that leukotrienes stimulate MGP secretion in the airways.[10,11] In human neutrophils, exogenous PAF induces additional synthesis of PAF, which requires activation of phospholipase A2.[22,23] The activation of phospholipase A2 could lead to release of arachidonic acid from the cell membrane, thus increasing production of prostaglandins and leukotrienes. Some studies showed that PAF influenced calcium homeostasis. Calcium uptake in rabbit platelets increased,[25] and calcium efflux in endothelial cells was stimulated by PAF.[26] It has been suggested that changes in the calcium content participate in regulation of eicosanoid biosynthesis. These findings suggest that PAF may induce MGP secretion from cultured chinchilla middle ear epithelial cells by activating arachidonate metabolism. Thus, we postulate that blockage of the lipoxygenase pathway leads to a reduction in MGP secretion in middle ear epithelial cells in vitro.

The current study demonstrated that supernatant MGP decreased significantly when cells were incubated with NDGA (a mixed inhibitor of both lipoxygenase and cyclooxygenase); this finding supports the notion that PAF-induced MGP secretion is related to arachidonic acid metabolites. This is consistent with our previous studies in which leukotriene B4 stimulated MGP secretion from cultured chinchilla middle ear epithelial cells. Unlike PAF receptor antagonists, NDGA is able to profoundly block MGP secretion. Experimental blockage of MGP secretion by inhibition of arachidonic acid metabolites provides a possibility for treatment of otitis media with effusion.

Cyclooxygenase metabolites have been shown to be responsible for MGP secretion in the intestinal tract.[27] This does not apply, however, to airway and middle ear epithelial cells in vitro. Lipoxygenase metabolites are much more active and important than cyclooxygenase metabolites in stimulation of MGP secretion in airway epithelium.[10,11] In the current study, the finding that cyclooxygenase inhibition did not significantly affect MGP secretion suggests that the autocrine prostaglandins induced by PAF in these cells might not play the same role in MGP secretion as they do in the intestinal tract. Pharmacologic concentrations of prostaglandins administered exogenously to middle ear epithelial cells, however, can enhance MGP secretion in vitro, as shown by Amesara et al.[28] This discrepancy reflects the way that prostaglandins act on MGP secretion. It may represent a concentration-dependent mechanism of prostaglandins acting on MGP secretion, whereby the PAF-enhanced production of prostaglandins is not high enough to trigger MGP secretion. Reaching such a conclusion, however, must exclude the possibility that prostaglandins stimulate MGP secretion via a nonspecific mechanism when a high concentration is administered. This is not currently understood. It is also unknown whether administration of a high concentration of prostaglandins activates more metabolic pathways intracellularly. One thing is certain: prostaglandin E2, one of the main products of the cyclooxygenase pathway seen in chinchilla middle ear fluid, did not stimulate MGP secretion in vitro, even at a high concentration.[28] Of interest, the mode of prostaglandin action on MGP secretion at different concentrations is similar to that of glucocorticoids on the inflammatory reaction. That is, high doses of glucocorticoids demonstrate an anti-inflammatory effect, while the physiologic concentration does not.

Lipoxygenase inhibition significantly decreased MGP secretion from the cells in baseline and PAF-stimulated states, indicating that the lipoxygenase metabolites, leukotrienes, play an important role in MGP secretion. A high level of MGP secretion may be expected from cells when the cyclooxygenase pathway is shut down. The theory is that in some tissues, shutting down one pathway enhances another; eg, shutting down the cyclooxygenase pathway drives arachidonic acids to the lipoxygenase pathway, thus increasing leukotriene production, which in turn enhances MGP secretion. Of interest, this did not occur in the current study. One explanation for this may be that during shutdown of the cyclooxygenase pathway, the arachidonic acids returned back to their precursors, rather than metabolizing to leukotrienes. This may explain why MGP secretion remained unaffected in cultured chinchilla middle ear epithelial cells.

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Ann Otol Rhinol Laryngol 105:1996

IMAGING CASE STUDY OF THE MONTH

POSTOPERATIVE CHOLESTEROL CYSTS OF THE MASTOID

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INTRODUCTION

One of the most common cystic lesions in the temporal bone is the cholesterol cyst. Cholesterol cysts slowly grow and expand into the middle or posterior cranial fossa, causing symptoms due to cranial nerve disturbance. Recently developed imaging techniques, however, have facilitated detection of silent cysts in the temporal bone. In this report, two cases of postoperative cholesterol cysts of the mastoid are presented, and diagnosis and treatment of this disease are discussed.

CASE REPORTS

Case 1. A 64-year-old woman complained of repeated otorrhea from the right ear with bilateral hearing loss and tinnitus. She had twice undergone open type tympanoplasty (modified radical mastoidectomy) for right chronic otitis media at another hospital, at the ages of 56 and 59. After surgery, tinnitus was treated in our clinic. In March 1993, the posterior part of the right mastoid cavity began to swell and develop a dark bluish color. Although she had no complaint, this swelling rapidly increased in size over 2 weeks and a brownish serous solution containing a crystal component was obtained by puncture, suggesting the existence of a mastoid cyst.

Therefore, computed tomography (CT) and magnetic resonance imaging (MRI) were performed. An axial CT scan showed a diffuse, expansile mass in the mastoid (Fig 1A). Ossicles were not detected. Axial T1- and T2-weighted MRI revealed a hyperintense expansile mass suggesting a cholesterol cyst in the mastoid (Fig 1B,C).

The mastoid cyst was exposed by postauricular incision and excised completely, together with the skin covering the cyst. Several small cysts were found around the cyst. There were numerous mastoid air cells that had not been exenterated by previous surgeries. These remaining cells were thoroughly eradicated. In the tympanic cavity, the stapes remained intact but had become separated from the tympanic membrane, suggesting so-called lateral healing of the eardrum. Modified type III ossicular reconstruction was performed by means of an incus homograft. The posterior wall of the external auditory canal was reconstructed with a piece of cortical bone, and the mastoid cavity obliterated by a Palva flap and hydroxyapatite granules.

Histopathologic examination showed cholesterol granuloma consisting of cholesterol clefts surrounded by fibroblasts and foreign body giant cells.

The postoperative hearing gain in the right ear was 30 dB on average.

Case 2. A 45-year-old man complained of right otorrhea and hearing loss. He had undergone a closed-type tympanoplasty in another hospital because of middle ear cholesteatoma at the age of 34. However, otorrhea had persisted for 11 years. Otoscopic examination revealed severe retraction of the eardrum and destruction of the posterosuperior bony wall of the external auditory canal. Debris of the cholesteatoma was visible in the attic.

Axial CT demonstrated a cystic mass in the mastoid region (Fig 2A). Ossicles were not detected. An MRI examination demonstrated a well-circumscribed mass with high signal intensity in the mastoid on both T1- and T2-weighted images, suggesting a cholesterol cyst (Fig 2B,C).

The mastoid cyst was exposed through a postauricular incision. The outer wall of the cyst was thickened and yellowish. After incision of the cyst, glistening brown serous fluid containing cholesterol crystals was noted and it was easy to completely remove the cyst. The aditus ad antrum showed bony closure. By drilling this bony structure, the attic was opened and

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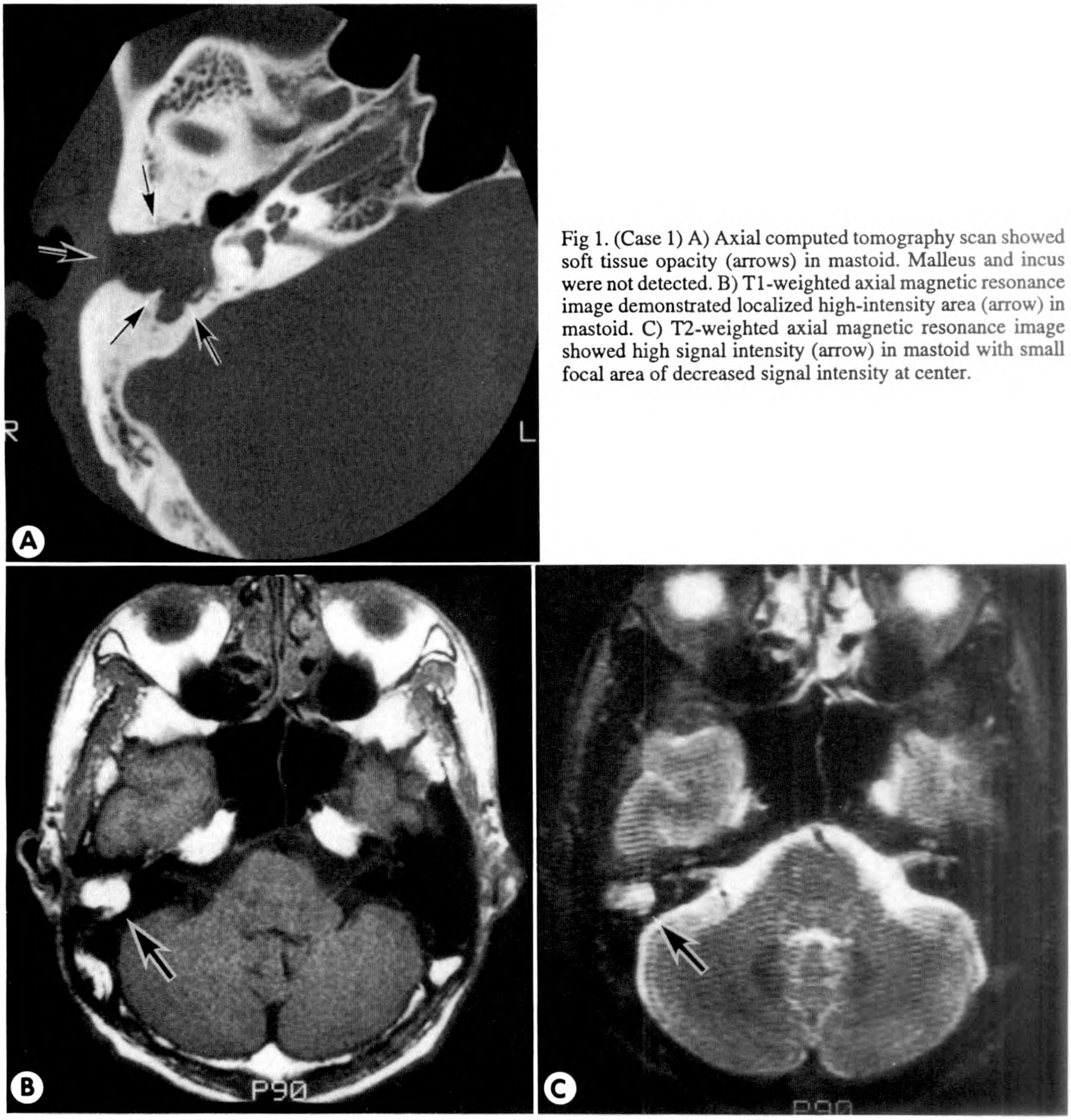

Fig 1. (Case 1) A) Axial computed tomography scan showed soft tissue opacity (arrows) in mastoid. Malleus and incus were not detected. B) T1-weighted axial magnetic resonance image demonstrated localized high-intensity area (arrow) in mastoid. C) T2-weighted axial magnetic resonance image showed high signal intensity (arrow) in mastoid with small focal area of decreased signal intensity at center.

the cholesteatoma removed. Staged surgery was selected because there was a possibility of residual cholesteatoma.

Histopathologic examination revealed a cholesterol cyst containing cholesterin crystals inside the fibrous connective tissue of the cyst wall.

One year later, the second stage of surgery was performed. The mastoid cavity was filled with granulation tissue, and a cystic lesion was found in the attic. There was no recurrence of the cholesteatoma. After removal of the pathologic lesions in the mastoid and attic, a modified type III tympanoplasty using an incus homograft was performed. The mastoid cavity was obliterated with hydroxyapatite granules to avoid a recurrence of the cyst.

DISCUSSION

Postoperative cholesterol cysts are rare among mastoid cysts.[1,2] There are various pathogeneses.[1] Thickening of the mucous membrane and formation of granulation tissue due to chronic inflammation, or new bone formation after middle ear surgery, can lead to the isolation of peripheral mastoid air cells (case 1) and closure of the aditus ad antrum (case 2). A cyst then develops and gradually enlarges to occupy the mastoid cavity. This type of cyst has a slow, insidious onset, which makes diagnosis quite difficult. The slow and silent expansion of the cyst may not be suspected until the bone is eroded and the cyst impinges on other structures such as the semicircular canal, facial nerve, or dura of the middle and posterior cranial fossa.[3,4] Therefore, periodic examinations are

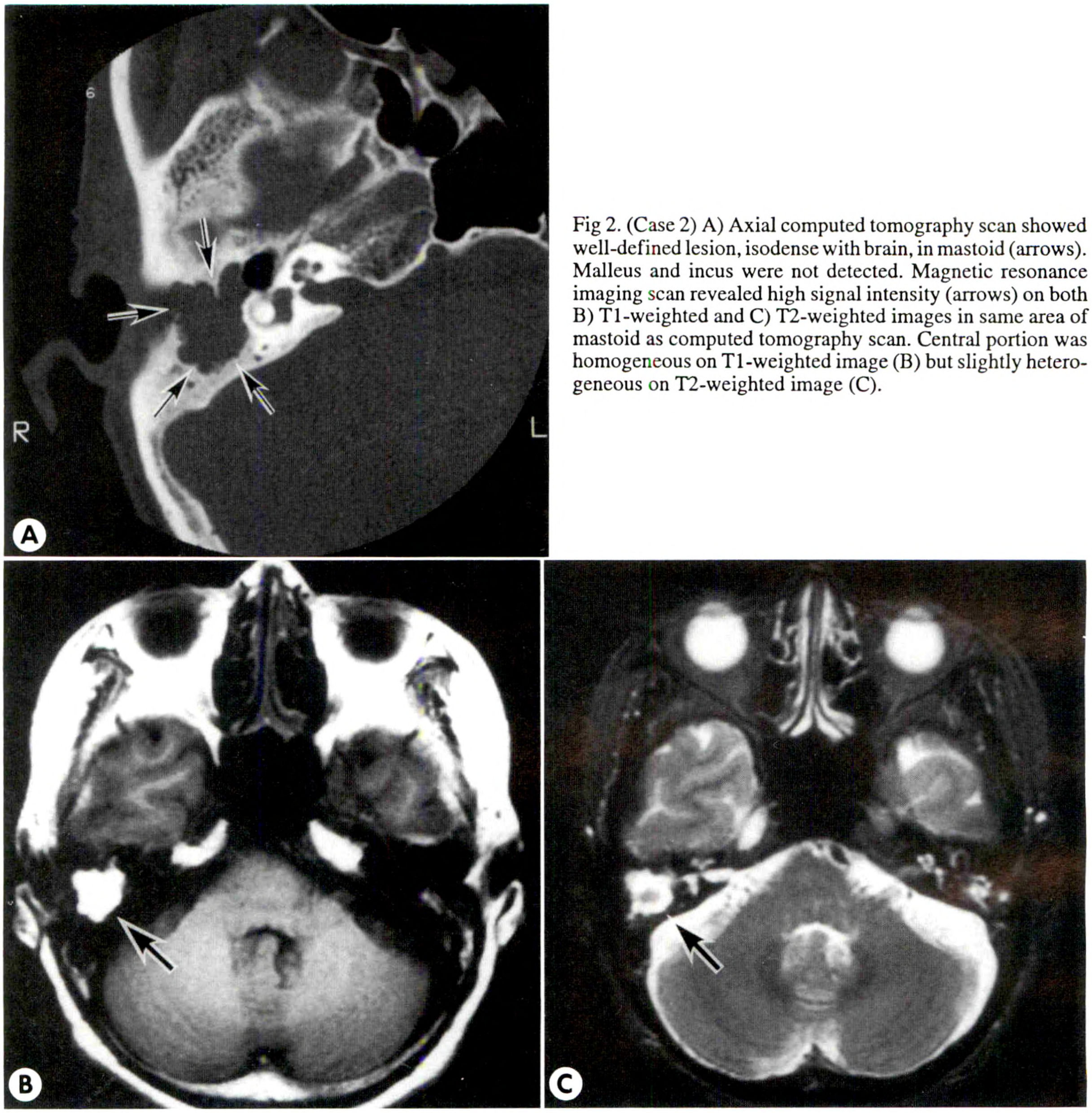

Fig 2. (Case 2) A) Axial computed tomography scan showed well-defined lesion, isodense with brain, in mastoid (arrows). Malleus and incus were not detected. Magnetic resonance imaging scan revealed high signal intensity (arrows) on both B) T1-weighted and C) T2-weighted images in same area of mastoid as computed tomography scan. Central portion was homogeneous on T1-weighted image (B) but slightly heterogeneous on T2-weighted image (C).

necessary to find postoperative cysts of the mastoid before otologic symptoms appear.

Cholesterol cysts can be identified on imaging studies such as CT and MRI. Computed tomography shows a round and low-density mass shadow similar to surrounding tissues.[3,4] In many cases, it is difficult to differentiate cholesteatoma only by CT findings.[5] Magnetic resonance imaging, however, shows very characteristic findings: the T1-weighted image shows a hyperintense mass only in cholesterol cysts, because breakdown products of hemoglobin after hemorrhage are rich in the cyst, while the T2-weighted image shows a hyperintense shadow both in cholesteatoma and cholesterol cysts.[2-8] From these characteristics, MRI is superior to CT in suggesting the specific diagnosis of cholesterol cyst.[2-7]

The treatment of cholesterol cysts consists of total excision and drainage surgery. In cases of petrous apex cholesterol cysts, total excision is considered unnecessary and is often not possible, because a cholesterol cyst has no distinct epithelial lining as is found in primary epidermoid cyst.[1,4] The cholesterol cyst in the mastoid region can be totally removed in some cases.[1] If permanent aeration is maintained for a long time, obliteration of the mastoid cavity is not required after removing the cyst. Mastoid obliteration might be needed, however, when the cavity becomes reisolated and results in recurrence of the cyst.

As the cyst in case 1 had rapidly increased in size, it was considered that the growth of the cholesterol cyst had been promoted by acute or subacute hemorrhage. To prevent a mastoid cyst, mastoid air cells should not be isolated from the tympanic cavity. In case 1, the remaining air cells of the mastoid were isolated due to modified radical mastoidectomy that had been performed 11 years earlier, and one of these cells developed rapidly, resulting in the mastoid cyst. If radical or modified radical mastoidectomy is selected, peripheral air cells should be thoroughly eradicated to prevent their isolation. In case 1, the remaining air cells were removed during the subsequent surgery and the wide mastoid cavity was obliterated after reconstruction of the posterior wall of the external auditory canal, because the patient had cavity problems such as otorrhea and crust formation in the mastoid.

In case 2, the mastoid cavity was isolated because the aditus ad antrum had closed due to new bone formation. This bony structure was removed by reoperation. However, there was a possibility of reclosure of the aditus postoperatively. We selected the mastoid obliteration technique, which is useful in preventing reclosure of the aditus and postoperative attic retraction pocket following recurrent cholesteatoma.

Concerning detection of recurrent lesions, postoperative follow-up by periodic radiologic examinations is essential.[8] An MRI scan is probably the best diagnostic choice for long-term follow-up evaluation of this lesion after surgical treatment and should be performed on a yearly basis — sooner, if clinical symptoms warrant reevaluation.[4,7]

In summary, we presented two cases of postoperative cholesterol cysts of the mastoid. Magnetic resonance imaging appears to be more specific than CT in establishing the preoperative diagnosis of cholesterol cyst. The importance of postoperative follow-up, including MRI examinations, after middle ear surgery is emphasized, because the cyst is asymptomatic until it impinges on other structures in and around the temporal bone.

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EDITOR'S NOTE — A variety of the above cysts were fairly common in postoperative nov-ovalis fenestration operations for otosclerosis in the mastoidectomy cavity, and called "blue-dome cysts" because of their similarity to those of the breast in females. They enlarged until secretional pressure came to equal the tensile strength of the skin and connective tissue over them, and remained stable for decades. No treatment was necessary unless they approached the rim of the fenestration. The reader not familiar with fenestration cavities should be aware of this. — *Brian F. McCabe, MD*

Ann Otol Rhinol Laryngol 105:1996

CLINICOPATHOLOGICAL CONSULTATION

SQUAMOUS NEOPLASTIC COMPONENT IN UNCONVENTIONAL SQUAMOUS CELL CARCINOMAS OF THE LARYNX

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Both otolaryngologists and surgical pathologists are involved in the diagnosis and treatment of lesions of the larynx that are best diagnosed as invasive squamous cell carcinomas of some specified degree of differentiation, not further subclassified (that is, conventional squamous cell carcinomas). On occasion, however, a patient will present with an invasive tumor that on histologic examination diverges from the expected pattern of a squamous cell carcinoma of the usual type and so raises the question of proper classification (on the part of the pathologist) and, following classification, a consideration of the prognostic and therapeutic implications of such a classification (on the part of the clinician). While some of these unconventional squamous cell carcinomas are rather indolent lesions (as, for example, the hybrid verrucous squamous cell carcinoma), others behave in a fashion similar to conventional squamous cell carcinomas (such as the adenoid squamous cell carcinomas), and yet others seem to behave more aggressively than do conventional squamous cell carcinomas of a similar size and stage (examples include the basaloid squamous cell carcinomas and adenosquamous carcinomas). Finally, the possibility exists within the larynx, as elsewhere in the body, that a nonepithelial lesion such as malignant melanoma may mimic a tumor more commonly encountered in that vicinity — namely, a squamous cell carcinoma — and so receive inappropriate treatment if such mimicry is not recognized.

INTRODUCTION

By a wide margin, the majority of malignancies arising in the larynx prove to be squamous cell carcinomas; most estimates place the incidence of squamous cell carcinoma at over 90% of all laryngeal malignancies. It has become apparent, however, that the traditional single category of squamous cell carcinoma actually includes several distinct oncotypes, which may, in some instances, follow a different course than do the usual squamous cell carcinomas. Moreover, there have now been recognized several nonsquamous tumor types that may be confused with laryngeal squamous cell carcinomas; this latter problem is particularly likely in the setting of limited biopsies in which treatment plans are constructed on the basis of histologic examination of only a very small portion of the entire lesion. Most otolaryngologists have had the experience of caring for patients whose initial biopsy diagnoses were later revised following a more detailed examination of a subsequent specimen by histochemical, immunohistochemical, or ultrastructural investigations.

Dividing the squamous cell carcinomas into several histologic subtypes is not an empty exercise in "splitting"; rather, each of these tumor types has its own degree of intrinsic malignancy that corresponds to the light microscopic appearance of the neoplasm — the prognosis, that is, is linked to the individual lesion's phenotype. A laryngeal basaloid squamous cell carcinoma, for example, appears to be a more aggressive lesion than does an ordinary squamous cell carcinoma of similar size.

In view of the fact that prognosis may vary as a function of the specific histologic phenotype of a given tumor, it should come as no surprise that the pretreatment staging evaluation also may vary among the different phenotypes (as, for that matter, may the definitive therapy). For example, only a limited workup is indicated for a diagnosis of verrucous squamous cell carcinoma, which is a decidedly indolent lesion; by contrast, the well-known propensity for small cell neuroendocrine carcinomas, atypical carcinoids, and basaloid squamous cell carcinomas to metastasize widely forces a more extensive staging procedure when these lesions are diagnosed. In the case of the latter group of (aggressive) lesions, the patient may be examined for distant metastases by modalities that might include endoscopic examination of the upper aerodigestive tract, chest radiography, whole lung tomography or computed tomography (CT) scanning, bone scintigraphy, liver ultrasound, iliac crest bone marrow aspiration and biopsy, CT of the head,

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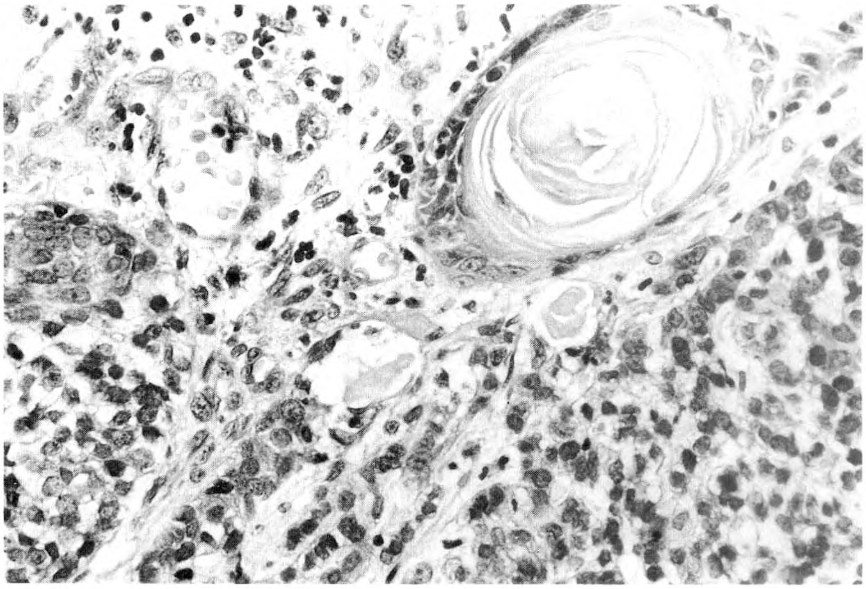


Fig 1. Basaloid squamous cell carcinoma of larynx showing two different components of lesion.

and blood chemistry determinations — depending upon the dictates of a particular case.

In the sections that follow, the chief phenotypes of the large family of squamous cell carcinomas, as well as some key differential diagnostic considerations, will be briefly described; in view of the constraints of space, these descriptions will not be comprehensive. The interested reader who desires more in-depth coverage of a particular type of lesion should explore the references at the end of this article.[1-34]

BASALOID SQUAMOUS CELL CARCINOMA

The first variant of squamous cell carcinoma to be considered here is the relatively rare *basaloid squamous cell carcinoma*. This tumor is characterized on physical examination as, most often, an exophytic mass lesion that may be ulcerated; that is, its physical characteristics do not diverge significantly from those of the conventional squamous cell carcinoma. On microscopic examination, however, this tumor is typified by the finding of a proliferation of recognizable islands of tumor cells with centrally located eosinophilic squamous cells intimately admixed with small, darker cells that are focally arrayed about the peripheries of the islands of tumor with their long axes perpendicular to the interface with adjacent fibrous tissue (that is, a palisaded or "picket fence" arrangement; Fig 1). The histologic difference translates into a clinical difference from conventional squamous cell carcinoma as well. In many series of basaloid squamous cell carcinomas of the head and neck region, a majority of patients have had identifiable regional nodal metastases at the time of initial evaluation, and a majority of patients ultimately developed distant metastases — an observation that has led most observers to believe that this is a more aggressive lesion than the commonly encountered squamous cell carcinomas of a similar size. Therapy, it has been suggested by some authors, might benefit from the inclusion of adjuvant therapy (such as chemotherapy) in addition to surgical excision, neck dissection, and radiotherapy.[5]

ADENOID SQUAMOUS CELL CARCINOMA

As is the case with many variants of squamous cell carcinoma, the *adenoid squamous cell carcinoma* does not usually suggest on physical examination that it may be a histologically different entity from the common squamous cell carcinomas. This lesion is most often seen in sun-exposed skin — particularly in the head and neck region — but has rarely been found to arise in deep sites, including the hypopharynx and the lung. Histologically, the superficial-most extent of this keratinizing squamous cell carcinoma exhibits an unusual degree of cystic change within islands of tumor cells; this cystic change appears to result from a loss of cohesion among the individual cells (acantholysis), and on occasion may be mistaken by the unwary for true glandular or vascular differentiation, which it is not (Fig 2). These tumors appear to behave in a fashion that parallels that of conventional squamous cell carcinomas of a like size and stage at presentation; thus, the import of recognizing this particular subtype of squamous cell carcinoma lies in its potential for confusion by the diagnostic surgical pathologist with other lesions, such as mucoepidermoid carcinoma and adenosquamous carcinoma, that may follow a divergent course from that of the usual type of squamous tumors (see below). Recommended therapy for adenoid squamous cell carcinomas does not differ from that for conventional squamous cell carcinomas, although it has

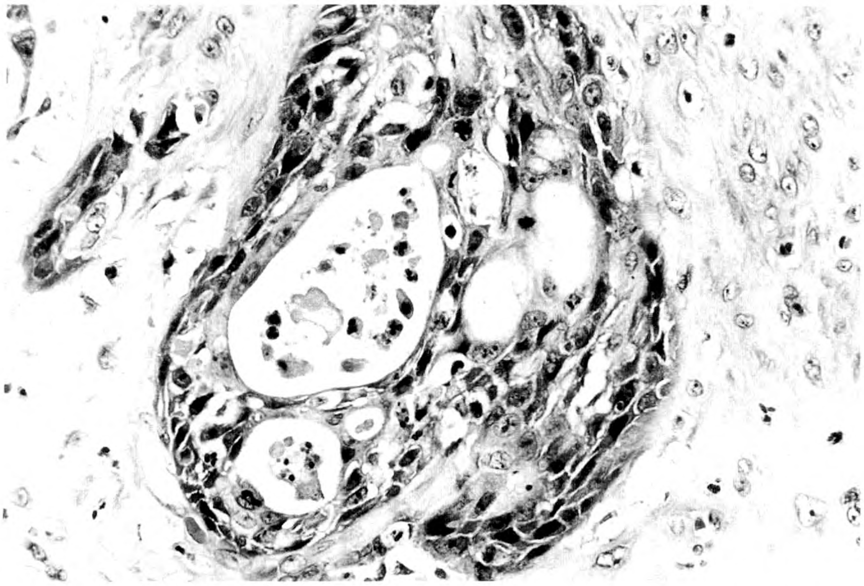


Fig 2. Adenoid squamous cell carcinoma of larynx producing pseudolumens containing cellular debris.

been suggested that (in contrast to the relatively indolent cutaneous lesions) *mucosal*-based adenoid squamous cell carcinomas of the head and neck region may be more aggressive lesions than their conventional squamous cell carcinomatous counterparts arising in the same region.[6-9]

ADENOSQUAMOUS CARCINOMA

When focal lumen formation is found in a biopsy otherwise suggestive of a conventional squamous cell carcinoma, the possibility of an *adenosquamous carcinoma* should always be considered. Again, these are lesions that are unlikely to be distinguished from their gross appearance alone. On light microscopy, the defining characteristic is the presence of both a malignant glandular component and a squamous component that are intimately admixed (and, as a consequence, differential diagnostic considerations include mucoepidermoid carcinoma and adenoid cystic carcinoma; Fig 3). These tumors are uncommonly encountered in the laryngeal region; they are highly aggressive tumors that are best approached by surgical excision with consideration given to the possibility of neck dissection. However, the utility of adjuvant therapy remains a matter of debate.[10-12]

MUCOEPIDERMOID CARCINOMA

The *mucoepidermoid carcinomas* are far more familiar to the salivary gland pathologist than to the laryngeal pathologist; nevertheless, these lesions are occasionally encountered in the laryngeal territory and so will sometimes present problems in diagnosis and treatment both for otolaryngologists and for surgical pathologists. On physical examination, these

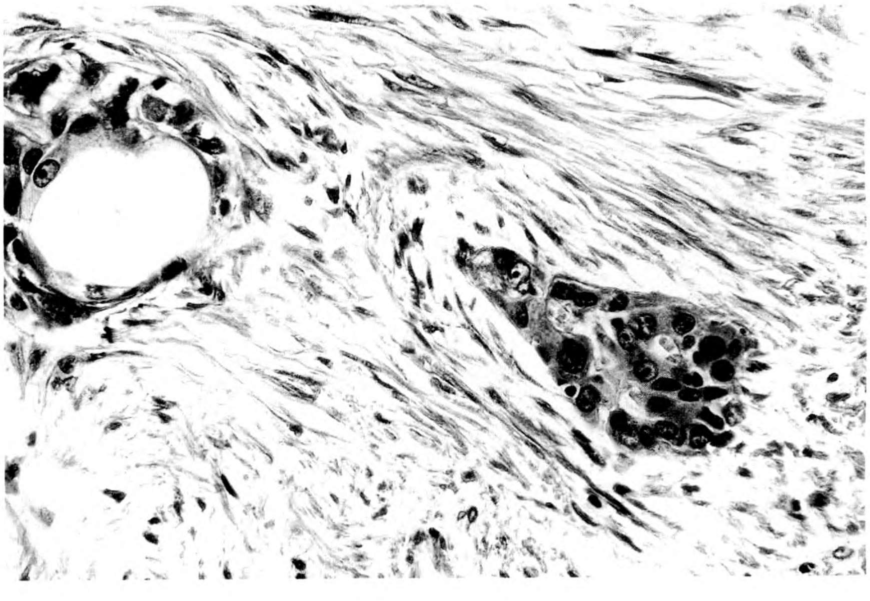


Fig 3. Adenosquamous carcinoma of larynx showing mixed squamous and glandular proliferation.

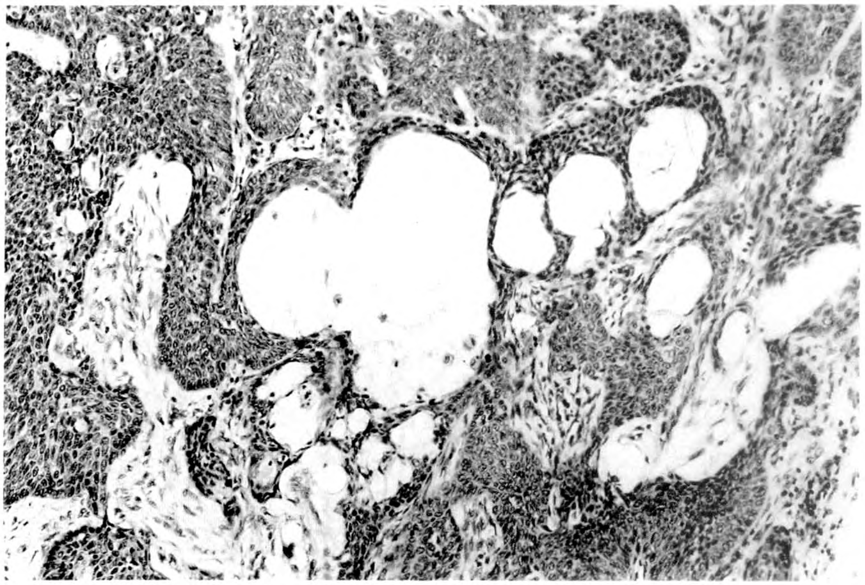


Fig 4. Mucoepidermoid carcinoma of larynx.

tumors are often firm and rubbery, often exhibiting a cystic appearance on cut section. The light microscopic hallmark of this tumor is an admixture of mucous cells, epidermoid cells, and intermediate cells (Fig 4). Positive histochemical stains for mucin can be useful in distinguishing mucoepidermoid carcinoma from pure squamous tumors (although it must be conceded that rare mucin vacuoles may be identified in some laryngeal squamous cell carcinomas — but the number of such vacuoles found in squamous cell carcinomas is significantly less than the number seen in true mucoepidermoid carcinomas). The mucoepidermoid carcinomas are members of the class of tumors that have in common the fact that their prognosis is closely correlated with their histologic grade. The low-grade mucoepidermoid carcinomas are rather indolent lesions for which complete local excision is usually adequate therapy; the high-grade tumors, by contrast, are best approached by radical surgical excision, neck dissection, and perhaps adjuvant radiotherapy as well.[13-15]

VERRUCOUS SQUAMOUS CELL CARCINOMA AND HYBRID VERRUCOUS SQUAMOUS CELL CARCINOMA

The warty, papillary appearance of an exophytic *verrucous squamous cell carcinoma* is readily recognized by most clinicians; this is a lesion that may be mistaken by the pathologist for a benign verrucous lesion on limited sampling. It may be only after several small biopsies have been (mis)interpreted as benign that the locally extensive nature of the tumor comes to light. The treatment of choice is excision (by laser or conventional surgery) without neck dissection. On occasion, however, the surgical pathologist will find on thorough sectioning of a suspected

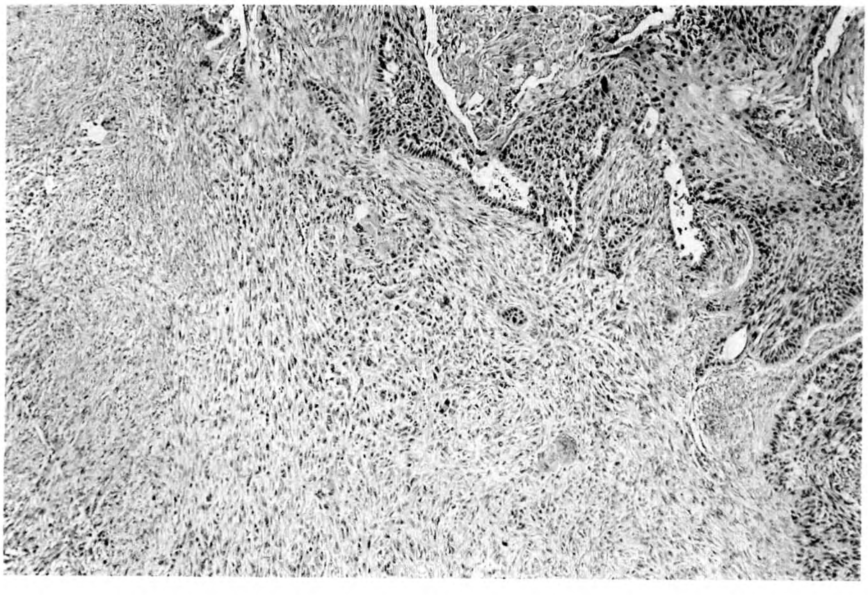


Fig 5. Spindle cell carcinoma of larynx. Bimorphic lesion comprising moderately well differentiated squamous cell carcinoma and sarcomatous component.

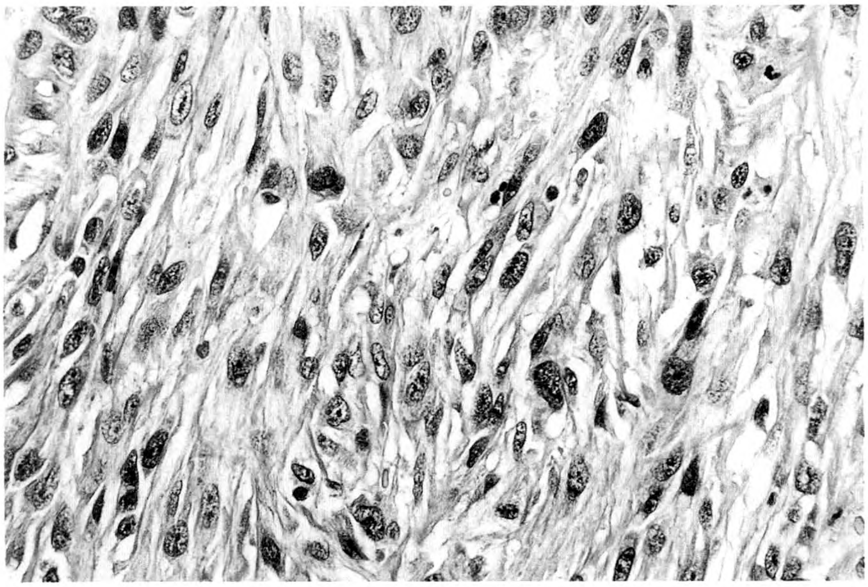


Fig 6. Spindle cell carcinoma of larynx showing pleomorphic spindle cells.

verrucous squamous cell carcinoma an unexpected component of such a lesion — namely, a focus of conventional invasive squamous cell carcinoma. Such a lesion, known as a *hybrid verrucous squamous cell carcinoma*, follows a relatively indolent course as well, but may possess some potential (albeit quite small) for spread to regional lymph nodes. This fact notwithstanding, the hybrid verrucous squamous cell carcinomas are probably best approached by local excision like that for the verrucous squamous cell carcinomas, without neck dissection unless these regions are clinically suspicious.[16-18]

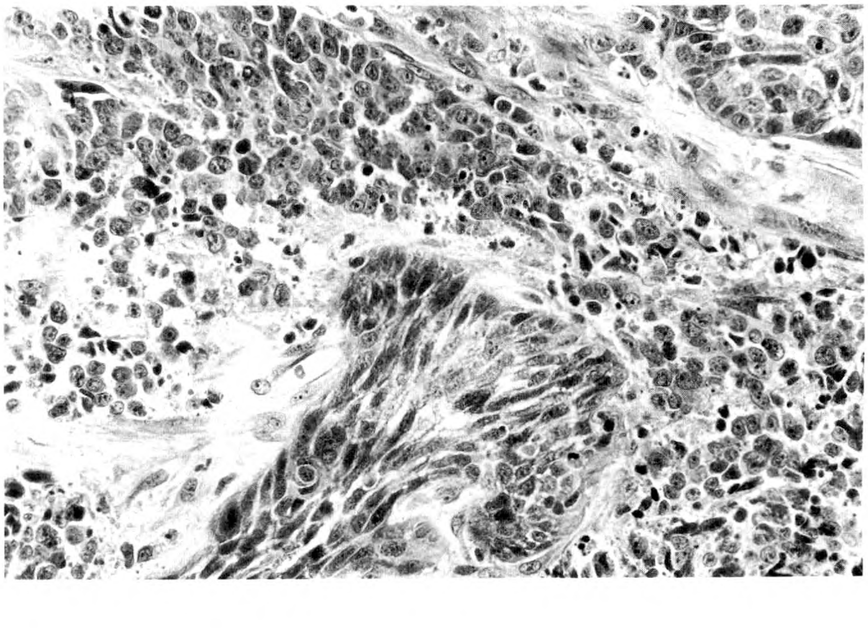
SPINDLE CELL (SARCOMATOID) CARCINOMA AND SQUAMOUS CELL CARCINOMA EXHIBITING DIVERGENT DIFFERENTIATION

The occasional patient will present with a large laryngeal lesion (most often a polypoid exophytic mass, projecting into the airway) that proves, on microscopic examination, to consist of both malignant epithelial and malignant mesenchymal components (Figs 5 and 6). Such tumors have been variously known as *sarcomatoid carcinomas*, spindle cell carcinomas, carcinosarcomas, metaplastic carcinomas, and pseudosarcomas. These are aggressive tumors with a poor prognosis, and so are best approached by attempts at complete surgical excision with neck dissection, with consideration given to the possibility of adjuvant irradiation.[19-23] Occasionally, squamous cell carcinomas of the larynx show the finding of pure squamous cell carcinoma associated with rhabdomyosarcomatous differentiation.[24]

NEUROENDOCRINE TUMORS

The family of *neuroendocrine tumors* is a heterogeneous one. One unifying factor, pertinent to the

Fig 7. Combined small cell neuroendocrine carcinoma of larynx.



SUBTYPES AND HISTOLOGIC MIMICS OF LARYNGEAL SQUAMOUS CELL CARCINOMA
Conventional squamous cell carcinoma (keratinizing and nonkeratinizing variants), not further subclassified
Basaloid squamous cell carcinoma
Adenoid squamous cell carcinoma
Adenosquamous carcinoma
Mucoepidermoid carcinoma
Verrucous squamous cell carcinoma and hybrid verrucous squamous cell carcinoma
Spindle cell carcinoma (sarcomatoid carcinoma)
Neuroendocrine tumors (atypical carcinoid, small cell neuroendocrine carcinoma of combined cell type and hybrid cell type)
Mimics of squamous cell carcinoma (malignant melanoma)

present discussion, is the potential for these tumors to contain areas (either as major or minor components) of cells with an abundance of eosinophilic cytoplasm — a finding that corresponds either to true keratinization (squamous change) or to a mimic keratinization (pseudosquamous change). In general, neuroendocrine neoplasms constitute a morphologically heterogeneous group of lesions that can be conveniently divided into two main groups: those of epithelial origin (typical carcinoid tumor, atypical carcinoid tumor, small cell neuroendocrine carcinoma) and those of neural derivation (paraganglioma). The combined small cell neuroendocrine carcinomas are neoplasms in which there is a defined component of small cell carcinoma (oat cell or intermediate type) together with squamous cell carcinoma (Fig 7) and/or adenocarcinoma. Atypical carcinoid tumor of the larynx and hypopharynx may be found adjacent to squamous cell carcinoma.[27,33] Dual neuroendocrine and epithelial differentiation may therefore occur in both organs. Immunohistochemistry is very important in the differential diagnosis vis-à-vis squamous cell carcinoma and neuroendocrine carcinoma of the larynx. Small cell neuroendocrine carcinoma of the larynx may reveal a squamous component, both in the combined cell type and in the hybrid cell type (a rare neoplasm that shows simultaneous evidence of squamous, glandular, neuroendocrine, and sometimes sarcomatous differentiation). Its distinction from squamous cell carcinoma of the usual type is facilitated by immunohistochemical and ultrastructural investigations. Treatment with chemotherapy followed by radiotherapy is recommended for this highly malignant neuroendocrine neoplasm. It is important for clinicians and pathologists to be aware of the existence of different types of neuroendocrine neoplasms of the larynx. These tumors indeed exhibit considerable differences in clinical behavior, from the extremely aggressive nature of small cell neuroendocrine carcinomas to the indolent course of typical laryngeal carcinoids, or the primarily benign behavior of paraganglioma; as a consequence of this, these individual tumor types demand that the attending clinicians devise different strategies tailored to the individual oncotype.[25-33]

OTHER TUMORS

Laryngeal *malignant melanomas* are among the tumor types that may show a pseudosquamous appearance, although neither keratinization nor intercellular bridges are found in melanomas.[34] It is in light of the existence of such mimics of conventional squamous cell carcinomas that the prudent surgical pathologist approaches a presumptive diagnosis of squamous cell carcinoma with a modicum of skepticism, wondering whether the lesion might actually represent a variant of squamous cell carcinoma of the usual type, or even a nonepithelial tumor masquerading as a carcinoma (see Table).

CONCLUSIONS

As is well known, a laryngeal biopsy may serve a multitude of purposes: the biopsy provides an initial diagnosis, of course, but it also may be factored into plans that are being made with regard to subsequent staging, treatment, and prognosis. The biopsy may take several forms, ranging from fine needle aspiration biopsy, to excision of a small intact tissue fragment with the aid of a cup forceps, to a complete resection of the lesion with clear margins (excisional biopsy). With only limited material for pathologic study, precise subclassification of a particular lesion may be impossible (or erroneous), and repeated biopsies may be needed. It is hoped that the foregoing discussion of several of the histologic subtypes and mimics of "ordinary" squamous cell carcinoma will provide a context in which decisions about the selection of a biopsy technique, the need for repeat biopsy, and the relevance of ancillary testing (as, for example, immunohistochemistry) can be intelligently made.

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TRACHEAL RESECTION WITH END-TO-END ANASTOMOSIS FOR ISOLATED POSTINTUBATION CERVICAL TRACHEA STENOSIS: LONG-TERM RESULTS

CRANIAL NERVE AND HEARING FUNCTION AFTER COMBINED-APPROACH SURGERY FOR GLOMUS JUGULARE TUMORS

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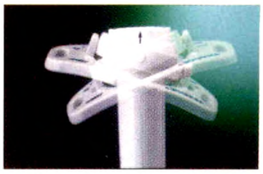

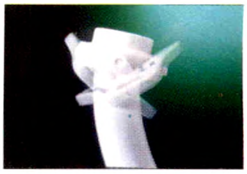

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1996 Annual Subscription Rates	*USA*	*International Surface Mail (All Regions)*
Institution*	$168.00	$188.00
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ANNALS OF OTOLOGY, RHINOLOGY & LARYNGOLOGY (ISSN 0003-4894) is published monthly by Annals Publishing Company, 4507 Laclede Avenue, St Louis, MO 63108. Second-class postage paid at St Louis, Mo. POSTMASTER: Send address changes to *ANNALS OF OTOLOGY, RHINOLOGY & LARYNGOLOGY*, c/o Annals Publishing Company, 4507 Laclede Avenue, St Louis, MO 63108.

Printed on acid-free paper

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ANNALS OF OTOLOGY, RHINOLOGY & LARYNGOLOGY

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VERTICAL PARTIAL LARYNGECTOMY WITHOUT TRACHEOTOMY: THE USE OF THE COOK AIRWAY EXCHANGE CATHETER TO AVOID TRACHEOTOMY

DALE H. RICE, MD
LOS ANGELES, CALIFORNIA

Occasionally, advances in another specialty provide the opportunity for an advance in otolaryngology–head and neck surgery. One example is the Cook airway exchange catheter. In very selected cases, the use of this exchange catheter will allow the performance of vertical partial laryngectomy without the need for tracheotomy.

KEY WORDS — Cook airway exchange catheter, tracheotomy, vertical partial laryngectomy.

INTRODUCTION

This is not for every patient, this is not for every surgeon, and this is not for every hospital. Traditionally, a vertical partial laryngectomy is performed in conjunction with a tracheotomy to provide an airway in the postoperative period. In the usual vertical partial laryngectomy, the resected hemilarynx is reconstructed with either a unipedicled or bipedicled muscle flap or with a mucosal flap and free fat graft. In either case, it is generally recommended that overcorrection be done to allow for future shrinkage. This overcorrection results in an inadequate airway that persists until edema and shrinkage have had time to occur.

A subgroup of vertical partial laryngectomy, the imbrication laryngectomy, as first described by Pleet et al,[1] is performed in such a way that there is much less edema and little or no overcorrection. This subgroup is amenable to this technique.

The Cook airway exchange catheter was devised to allow a change from one endotracheal tube to another without the necessity of performing a laryngoscopy to visualize the larynx.

The Cook airway exchange catheter is a rather stiff but flexible tube that is hollow, with multiple perforations at one end and a *removable* universal adapter at the other end. It comes in a variety of sizes for all ages. If one wishes to change the endotracheal tube in an intubated patient, this exchange allows it to be done in any setting without the necessity of laryngoscopy or visualizing the larynx. First, an exchange catheter of appropriate size is passed through the en-

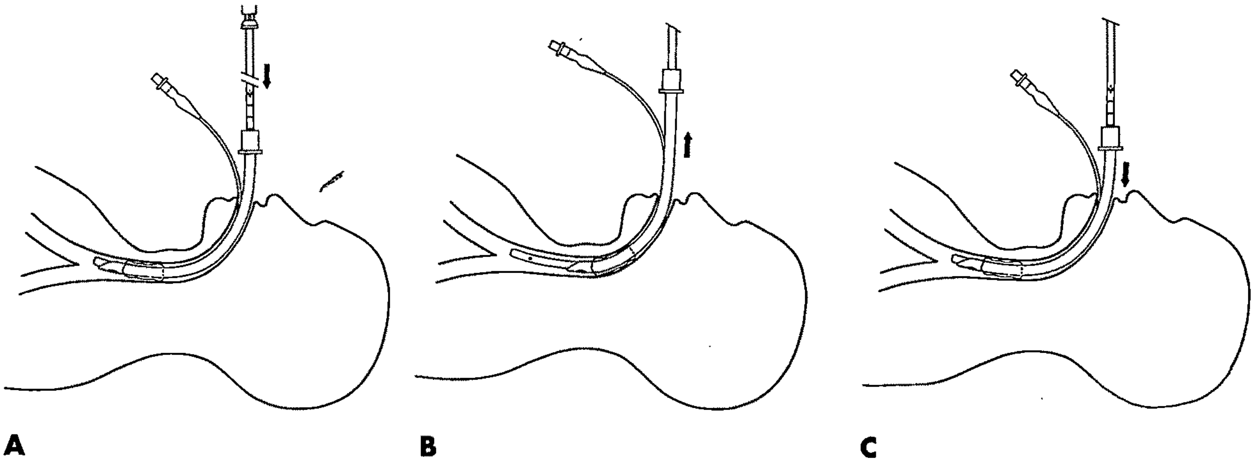

Fig 1. Technique. A) Cook airway exchange catheter is inserted through endotracheal tube. B) Endotracheal tube is removed, leaving Cook airway exchange catheter in place. C) New endotracheal tube is advanced over Cook airway exchange catheter until properly placed.

From the Department of Otolaryngology–Head and Neck Surgery, University of Southern California, Los Angeles, California.

Presented at the meeting of the American Laryngological Association, Palm Desert, California, April 29-30, 1995.

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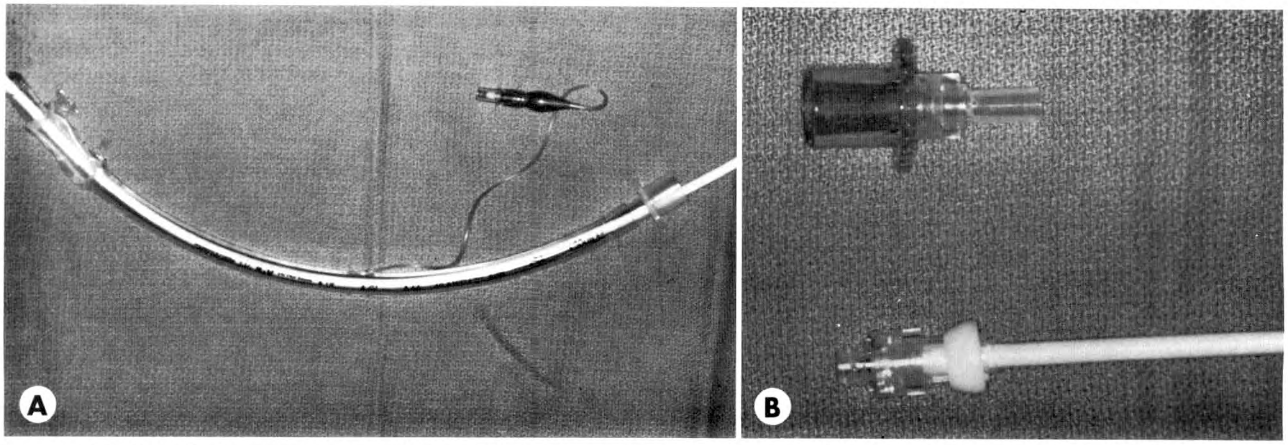

Fig 2. Cook airway exchange catheter. A) Catheter passing through endotracheal tube. B) Adapter at end of catheter.

dotracheal tube in the patient until it is in the trachea (Fig 1A). If necessary, the patient can be ventilated through the exchange catheter by means of the universal adapter. Once the replacement tube is available, the universal adapter is removed. The indwelling endotracheal tube can then be removed over the exchange catheter (Fig 1B) with the catheter left in the trachea. The replacement tube is then passed blindly over the exchange catheter until it is properly positioned (Fig 1C). The exchange catheter is then removed.

TECHNIQUE

This technique is used only in patients undergoing imbrication vertical laryngectomy. The patient is anesthetized and then intubated nasotracheally with the smallest tube compatible with successful ventilation, usually a 6.5-mm tube. The imbrication laryngectomy is performed in the standard fashion,[2] and the patient is left intubated.

On the morning of the third postoperative day, a small Cook airway exchange catheter (Fig 2) is inserted through the nasotracheal tube into the trachea, and the nasotracheal tube is backed out of the larynx into the hypopharynx, while the exchange catheter is left in the trachea. The patient is then allowed to breathe normally and monitored carefully.

Thus, the patient is breathing normally while the nasotracheal tube sits in the pharynx above the larynx. The Cook airway exchange catheter passes through the endotracheal tube into the trachea. A small pediatric-sized exchange catheter is used for two reasons. First, only a small exchange catheter can successfully negotiate the bends in a nasotracheal tube. Second, a small catheter gives the patient more room around it to breathe. Moreover, if desired, cool, humidified air can be delivered through the catheter by connecting it to the universal adapter. With the catheter in place in the trachea, should the patient develop any airway compromise, the nasotracheal tube could be advanced blindly back into the trachea.

In no instance to date has a patient needed reintubation. The endotracheal tube is left in the hypopharynx with the exchange catheter still in the trachea for 24 hours while the patient is observed. At the end of this time, the nasotracheal tube and the Cook airway exchange catheter are removed together, and the patient is started on a clear liquid diet. During this time the patient can be managed in any unit of the hospital capable of caring for a nasotracheal tube. I have not compared the cost of 3 days of nasotracheal intubation to the costs in operating time, equipment, and care of a tracheotomy, but expect it would be similar.

RESULTS

To date, this technique has been used on six carefully selected imbrication laryngectomy patients. In no instance has a patient needed to be reintubated or had any other respiratory or swallowing problem. In all cases, the patient has been discharged by the sixth postoperative day, eating a normal diet, with a satisfactory airway.

CONCLUSION

In carefully selected patients who are to undergo an imbrication laryngectomy, the procedure may be performed without performing a tracheotomy, by using the above-described technique. A combination of nasotracheal intubation and the careful use of the Cook airway exchange catheter allows the patient to be maintained on nasotracheal intubation for 48 hours postoperative and then extubated in a manner that is quite safe and that would allow ready reintubation without laryngoscopy and without significant trauma, should the need arise. In this small series, no patient has needed reintubation, and all have done well.

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PEDIATRIC OTORHINOLARYNGOLOGY

The Seventh International Congress of Pediatric Otorhinolaryngology will be held in Helsinki, Finland, June 7-10, 1998. For more information, contact Pekka Karma, MD, Chairman, or Hans Ramsay, MD, Secretary, ENT Department, Helsinki University Central Hospital, Haarmaninkatu 4 E, FIN-00290 Helsinki, Finland; telephone 358 9 4713019; fax 358 9 4715010; E-mail ramsay@hansram.pp.fi.

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FOURTH ANNUAL GOLF SEMINAR — ADVANCES IN OTOLARYNGOLOGY–HEAD AND NECK SURGERY

The Fourth Annual Golf Seminar — Advances in Otolaryngology–Head and Neck Surgery will be held March 11-16, 1997, at the Pinehurst Resort & Country Club in North Carolina. For information, contact Leslie Sullivan, Coordinator, University of Washington School of Medicine Virginia Merrill Bloedel Hearing Research Center, Box 357923, Seattle, WA 98195-7923; E-mail sullivan@u.washington.edu; telephone (206) 685-2962; fax (206) 616-1828.

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GROWTH FACTORS IN SUBGLOTTIC STENOSIS

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We sought to define the role of fibrogenic peptides in subglottic stenosis (SGS). Biopsy specimens were obtained from patients with stenosis following endotracheal intubation (group 1, n = 5, mean age 5), patients without a history of any precedent trauma, ie, idiopathic stenosis (group 2, n = 3, mean age 40), and those without stenosis (group 3, n = 3, mean age 70). Formalin-fixed biopsy specimens were analyzed following immunohistochemical staining to determine if epidermal growth factor (EGF), platelet-derived growth factor–AA and -BB (PDGF-AA/BB), transforming growth factor–β1 and –β2 (TGF-β1,β2), or basic fibroblast growth factor (*b*FGF) was deposited in these tissues. Blinded analysis revealed TGF-β2 and PDGF-AA to be present in seven of eight biopsy specimens from SGS and absent in controls. Staining for PDGF-BB was observed in the mucosa and submucosa and occasionally within vessel walls. Staining of individual growth factors appeared to correlate closely with the presence of granulation tissue. Essentially no *b*FGF or TGF-β1 was observed. Differences were found between patients in groups 1 and 2; tissue from group 1 revealed deposition of EGF and PDGF-BB in submucosa, epithelium, and vasculature. In summary, our experimental findings implicate PDGF and TGF-β2, perhaps acting in concert, in mediating the pathologic fibrotic process observed in subglottic stenosis. Epidermal growth factor, in conjunction with TGF-β and PDGF, may also have a role, but further investigation is needed to more precisely define it.

KEY WORDS — fibrogenic peptide, growth factor, subglottic stenosis.

INTRODUCTION

Subglottic stenosis (SGS) remains a challenging, complex clinical problem, the cause of which is poorly understood. In the early to middle part of this century, SGS was frequently a sequela of infection or trauma. More recently, endotracheal intubation, while providing requisite respiratory support, has precipitated many cases of acquired laryngotracheal stenosis. The reported incidence of SGS following endotracheal intubation is 1% to 9%,[1,2] although confounding variables such as endotracheal tube size, duration of intubation, and the number of reintubations may significantly influence the true incidence.[3] In addition, some children are born with a narrowed subglottic lumen (congenital SGS), and rarely, patients may develop stenoses without prior airway manipulation (idiopathic SGS).[4] In each of these situations the pathologic findings are identical, with evidence of abundant extracellular matrix deposition distorting the normal architecture.

In the past several years, the regulation of growth factors in abnormal tissue repair processes has come under intense investigation.[5] This has resulted in the accumulation of a large body of information, but to date, we are unaware of any reports implicating these factors in the pathogenesis of upper airway stenoses. Growth factors are polypeptides that induce the proliferation, differentiation, and synthetic activity of a variety of cells. We chose to study several well-characterized growth factors that appear relevant to tissue reparative processes, including epidermal growth factor (EGF), platelet-derived growth factor (PDGF), transforming growth factor–β (TGF-β), and basic fibroblast growth factor (*b*FGF). Utilizing immunohistochemical staining, we provide evidence indicating that specific peptides are deposited in postintubation airway stenosis or idiopathic SGS. Similar staining patterns were observed regardless of the underlying cause, suggesting common pathogenic pathways. This is the first report to our knowledge that implicates growth factors in the evolution of SGS, and provides a mechanistic framework to guide future treatment strategies.

METHODS

TISSUE SPECIMENS

Subglottic biopsies of clinically active sites were obtained from three groups of subjects. Group 1 consisted of five patients with various degrees of acquired stenosis associated with a history of previous intubation, and group 2 consisted of three patients without a history of any precedent trauma (idiopathic stenosis). The clinical information concerning these patients is summarized in Table 1. Each biopsy specimen was obtained under general anesthesia at

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TABLE 1. PATIENT CLINICAL PRESENTATIONS

Patient No.	*Age*	*Sex*	*Clinical Description*	*Pathologic Description*	*Diagnosis*
Group 1					
1	8	M	Stenosis after intubation for head injury with 90% lumen obliterated (no laser)	Immature granulation tissue, minimal fibrosis	Secondary SGS
2	2	F	26-week premature infant intubated for 2 months with 80% lumen obliterated (no laser)	Mature granulation tissue with scar formation and extensive fibrosis	Secondary SGS
3	9	M	1 year status post burn injury and intubation with 60% lumen obliterated (no laser)	Avascular fibrotic tissue with extensive fibrosis	Secondary SGS
4	1.5	F	24-week premature infant with tracheal stenosis with 90% lumen obliterated (multiple laser procedures)	Immature granulation tissue, mild fibrosis	Secondary SGS
5	6	F	4-year history of stenosis after caustic ingestion and intubation, 70% lumen obliterated (2 laser procedures)	Immature granulation tissue, minimal fibrosis	Secondary SGS
Group 2					
6	36	F	Status post multiple laser procedures (50% lumen obliterated)	Moderate fibrosis, with focus of acute and chronic inflammation	Idiopathic SGS
7	38	F	Status post multiple laser procedures (50% lumen obliterated)	Moderate fibrosis	Idiopathic SGS
8	46	F	Status post multiple laser procedures (50% lumen obliterated)	Maturing granulation tissue, mild fibrosis	Idiopathic SGS
Group 3					
9	75	M	Postmortem, no history of airway disease	Normal subglottic mucosa, no significant fibrosis	Control tissue
10	75	M	Postmortem, no history of airway disease	Normal subglottic mucosa, no significant fibrosis	Control tissue
11	60	M	Postmortem, no history of airway disease	Normal subglottic mucosa, no significant fibrosis	Control tissue

SGS — subglottic stenosis.

the time of surgical intervention. Group 3 consisted of macroscopically normal subglottic mucosal tissue from three randomly selected postmortem cases for comparison with the patient samples. The biopsy specimen was taken at the level of the first tracheal ring. These individuals did not have a history of either intubation or barotrauma.

ANTISERA

The primary antibodies were affinity-purified monoclonal (*b*FGF and EGF) or polyclonal antibodies (TGF-β1, TGF-β2). They were obtained from Research and Development Systems (Minneapolis, Minn; TGF-β1, TGF-β2, and *b*FGF), Genzyme Corporation (Boston, Mass; PDGF-AA and PDGF-BB), or Oncogene Science (Manhasset, NY; EGF).

STAINING PROCEDURE

Sections (5 μm) from formalin-fixed, paraffin-embedded blocks were then floated in a 45°C water bath, placed onto Sobo-coated slides (Sobo glue, Slomons, Newark, NJ), warmed until dry, and then placed into a 58°C dry oven for 1 hour.

The tissue sections were deparaffinized and hydrated by standard histologic methods. The sections were treated in 3% hydrogen peroxide in water for 20 minutes to quench endogenous peroxidase activity. The slides were then briefly rinsed repeatedly in phosphate-buffered saline (PBS) pH 7.2 (Difco Laboratories, Detroit, Mich) at room temperature. Immunohistochemical staining was performed by a labeled avidin-biotin technique employing diaminobenzidine as a substrate. Sections were stained directly with primary antisera for TGF-β2, PDGF-AA, and PDGF-BB without any prior enzymatic digestion techniques. To obtain the optimal stain for each of the growth factors in formalin-fixed tissue, a variety of enzymes were employed under different conditions to digest the tissue and expose the antigenic epitopes. To expose the epitopes for the primary antibody, serial sections were pretreated by digestion with 0.1% hyaluronidase for 30 minutes at 37°C. The exposure of TGF-β1 epitopes required treating the sections with hyaluronidase as above and then with 0.1% Pronase (Calbiochem, La Jolla, Calif) for 5 minutes at 37°C for maximal staining. The tissue staining for PDGF-AA and PDGF-BB was optimized by treatment with pepsin 0.1% for 30 minutes at 37°C. All of the sections were treated with the appropriate blocking serum, blotted, and incubated overnight with various

TABLE 2. GROWTH FACTOR STAINING IN BIOPSY SPECIMENS OF SUBGLOTTIC STENOSIS

Patient No.	*TGF-β2*	*EGF*	*PDGF-AA*	*PDGF-BB*
1	0	0	0	0
2	E,V,S	0	S,E	s,E,V
3	s	S	s,e	s,e,v
4	E	s,V	E	E,V
5	S,e	S,e	S,e	s
6	S	s	s,E,v	0
7	s	0	s,V	0
8	s	0	s,e	0
9	0	0	0	0
10	0	0	0	0
11	0	0	0	0

No significant staining of TGF-β1 or *b*FGF was observed.

Lowercase letters — mild staining (1 in text), uppercase letters — extensive staining (2 in text), 0 — no staining. (E,e — epithelium, V,v — vasculature, S,s — submucosa.)

dilutions of primary antisera at 40°C. At room temperature the slides were rinsed in PBS for 5 minutes three times. After incubation with the appropriate biotinylated secondary antibody (Vector Laboratories, Burlingame, Calif) for 30 minutes, the slides were washed again in PBS as above. The slides were inoculated with streptavidin-peroxidase (Vector Laboratories) and washed as above. The specimens were reacted with 30% hydrogen peroxide in 0.5 mg/mL diaminobenzidine (Polysciences, Warrington, Pa) for 12 minutes at room temperature. The slides were rinsed in running tap water for 10 minutes and counterstained for 1 minute with hematoxylin (Sigma, St Louis, Mo), dehydrated, and mounted.

The slides were blindly analyzed by an experienced immunopathologist (F.M.) using a semiquantitative scale depicting the degree of peroxidase staining (0 = absent; 1 = mild; 2 = extensive). Adjacent serial sections from each biopsy sample were prepared by standard techniques with hematoxylin and eosin for routine histology and Masson's trichrome stain to assess matrix deposition. The hematoxylin and eosin slides were evaluated separately for any inflammatory component and to depict the stage of the reparative process in each specimen. The trichrome-stained specimens were scored on the scale described above to monitor the degree of SGS present.

RESULTS

CLINICAL DATA

The first five patients in group 1 studied in this series were intubated for various lengths of time ranging from 4 weeks to 5 months. Patients 6 through 8 from group 2 had no history of intubation, but at the time of biopsy underwent laryngotracheal reconstruction after failure of endoscopic laser therapy. Samples 9 through 11 (group 3) were obtained, as controls, after these individuals died. A clinical summary of patients from groups 1, 2, and 3 is shown in Table 1.

MICROSCOPIC ANALYSIS

The hematoxylin and eosin staining revealed regions of edema, angiogenesis, inflammation, and squamous metaplasia. The trichrome-stained sections revealed considerable variation in the extent of fibrosis among cases (Table 1). We were unable to find a significant correlation between the extent of fibrosis and the deposition of any specific growth factor.

IMMUNOHISTOCHEMICAL STAINING

Extensive analysis comparing the various enzyme pretreatments resulted in the following conclusions, pertinent in obtaining optimal staining when utilizing *formalin-fixed* respiratory mucosal tissue. Slightly different processing techniques were deemed necessary for each growth factor of interest. Maximal staining for PDGF-AA and PDGF-BB requires pepsin pretreatment, TGF-β staining requires pretreatment with hyaluronidase, and EGF staining is generally not enhanced by any specific pretreatment.

Staining patterns were examined for each growth factor deposited within the submucosa and within the epithelium when possible, and the vascular structures were separately analyzed for involvement. The results for each are presented as follows.

Submucosa. The results of immunohistochemical staining for the presence and distribution of TGF-β2, EGF, PDGF-AA, and PDGF-BB after tissue digestion with hyaluronidase or pepsin are summarized in Table 2. Staining for *b*FGF and TGF-β1 following enzyme pretreatment was essentially negative and therefore not included in Table 2. Strong positive staining was found for TGF-β2, EGF, and PDGF-AA, when compared to normal respiratory submucosal tissue. The deposition of TGF-β2 was found in the same vicinity and to the same intensity as PDGF-AA, reflecting a high degree of correlation. Less prominent was the EGF staining, even under optimal conditions. PDGF-BB was detectable in group 1, but no staining was found in groups 2 and 3. Group 1 could be distinguished by more extensive deposition of both PDGF-BB and EGF, more prominent in some cases than others.

Vasculature. The results of the immunohistochemical staining for presence and distribution of PDGF, EGF, *b*FGF, and TGF-β in the microvasculature are also shown in Table 2. Pretreatment of serial sections with different enzymes did not result in significant differences. The vessels frequently stained positive for TGF-β2, PDGF, and EGF in a finely dispersed pattern, although consistent staining was not observed in either the acquired or idiopathic SGS samples. We found PDGF-BB more commonly in group 1 than in group 2. Fibroblast growth factor was not demonstrable in any of these samples, despite its putative role in angiogenesis.

Epithelium. In comparing the diseased specimens with the normal architecture by means of routine hematoxylin and eosin staining, it was evident that the control epithelial tissue was composed of respiratory epithelium while the patient biopsy samples in group 1 revealed squamous metaplasia. In group 2, none of the idiopathic stenoses were positive for epithelial staining. However, two thirds of these samples lacked squamous metaplasia. Four of the five postintubation samples (group 1) had multiple growth factors present (Table 2). The pattern of the staining however, was not consistent throughout these samples, although PDGF was more prominent in group 1 than in group 2.

Blinded independent observers (F.M., B.L.G.) were in close agreement scoring the intensity of staining. Representative staining patterns for the various growth factors are shown in the Figure. The Figure, A, demonstrates a patient (No. 5) with EGF distributed diffusely in the extracellular matrix of the subepidermal mucosal tissue, with essentially no staining within fibroblasts or vascular structures. In comparison, an absence of staining was found in tissue treated without the primary antibody as a control for nonspecific background staining (see Figure, B). Most of the specimens demonstrated minimal staining for TGF-β1, whereas PDGF-AA deposition was generally quite striking throughout the matrix and within the fibroblasts (see Figure, C). The PDGF staining was in contrast to TGF-β2 staining, which was prominent throughout the matrix but barely detected within cytoplasm of fibroblasts by the techniques employed (see Figure, D). The vasculature was strongly stained by antibody to PDGF-BB, as observed in tissue from patient 4 (see Figure, E), with mild staining throughout the matrix. Of interest, it appeared that the staining of PDGF-BB within the vasculature spared the luminal endothelium and stained the vascular walls more prominently (see Figure, E).

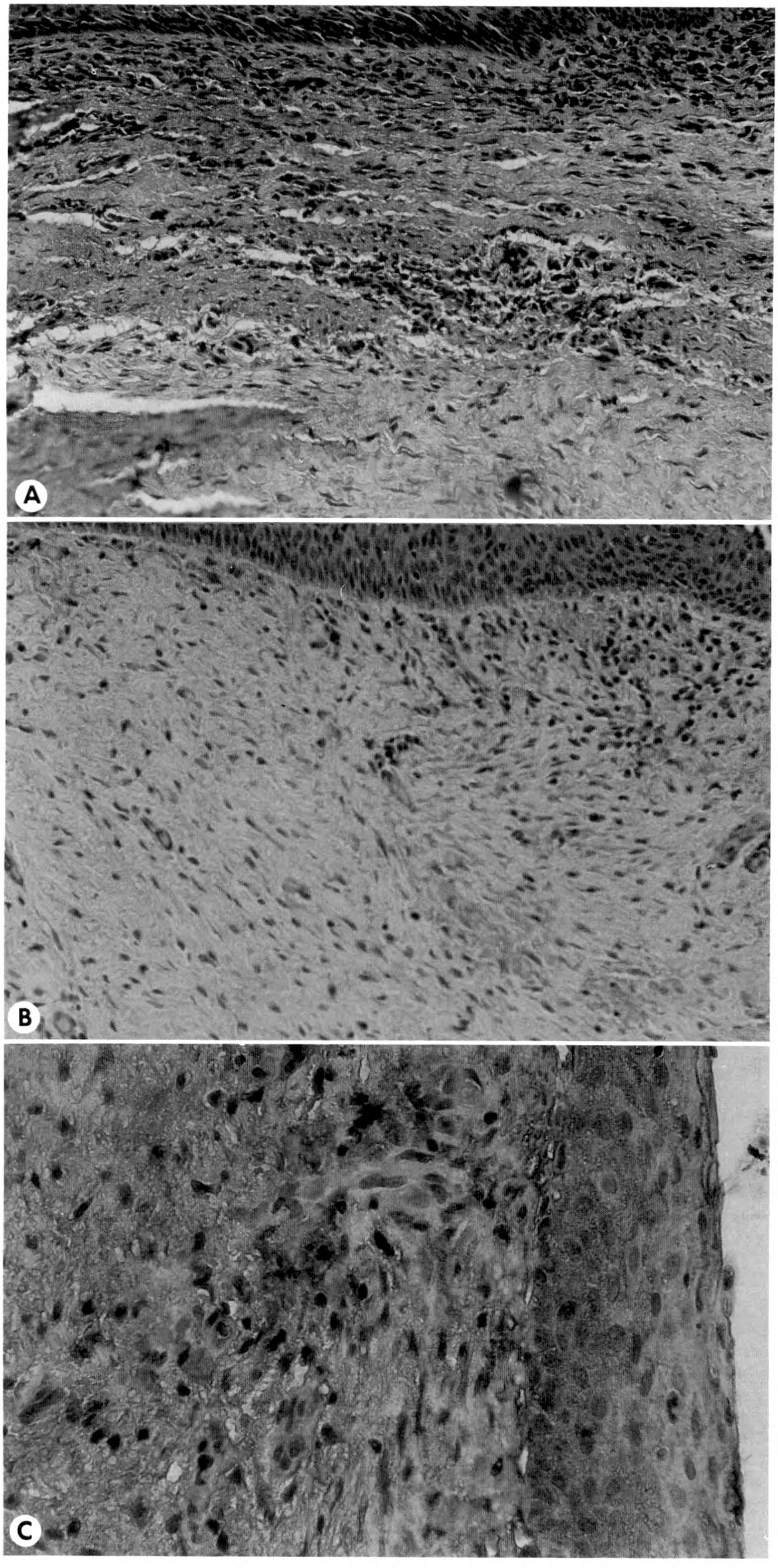
DISCUSSION

Subglottic stenosis can be characterized by the development of excessive scarring following an identifiable tissue insult such as prolonged intubation or, less commonly, without any precedent event, as in idiopathic SGS.[4] The precise pathogenesis of this disease and other fibrosing disorders remains unknown. However, recent studies have emphasized the role of peptide growth factors in numerous other fibrotic diseases, eg, systemic sclerosis, pulmonary fibrosis, hepatic cirrhosis, and nephrosclerosis.[5-7] Hence, we performed a retrospective analysis using formalin-fixed tissue biopsies to determine if growth factors are involved in the development of SGS. We studied growth factors from four different families, as represented by EGF, PDGF, TGF-β, and *b*FGF.

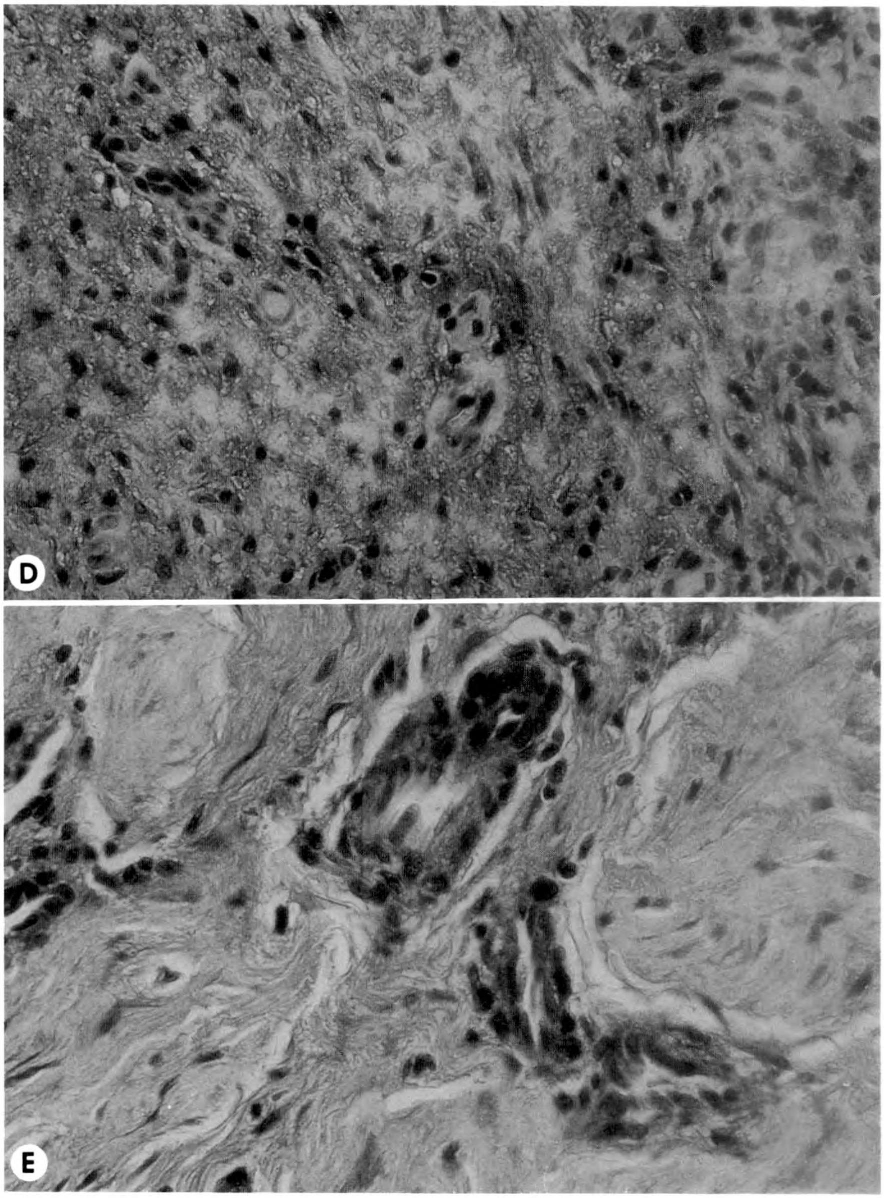
Our study demonstrated that these growth factors are prevalent in tissue from patients with evolving SGS, possibly implicating a role in the pathogenesis. In particular, staining for TGF-β2 and PDGF-AA was found in seven of eight biopsy samples, while it was absent in the healthy controls. Staining for PDGF-BB was observed within the mucosa (three of eight samples), within the submucosa, and occasionally within vessel walls (two of eight samples). Epidermal growth factor and PDGF-BB were found in association with the combination of TGF-β2 and PDGF-AA in the submucosa where fibrosis was noted on the trichrome stain. Group differences were noted, in that group 1 appeared to have more extensive deposition of EGF and PDGF-BB as compared to the other groups. No staining of TGF-β1 or *b*FGF was observed in any patients.

The staining of individual growth factors appeared to correlate most closely with the presence of granulation tissue. For example, patient 5 had minimal fibrosis observed on the biopsy, but had an abundance of growth factors at an early stage in the formation of the granulation tissue. Patient 5 subsequently demonstrated clinical evidence to suggest the maturation of a progressive scarring process involving the airway. This eventually necessitated carbon dioxide laser excision for respiratory compromise. Patients 3 and 4 showed extensive fibrosis but scant staining for the presence of TGF-β and PDGF. As the narrowing process matures, it appears that growth factor signals decrease as an avascular fibrotic tissue replaces the normal architecture.

Platelet-derived growth factor is a highly cationic 30 kd protein composed of two subunits A and B, linked by disulfide bonds.[8] It exists in three dimeric isoforms: AB, BB, or AA heterodimer or homodimer. Initially isolated from platelet alpha-granules, but also secreted by smooth muscle cells, endothelial cells, fibroblasts, and activated macrophages, PDGF activates the proto-oncogene, c-myc,[9] which is capable of initiating early genetic events leading to DNA replication and cell division and enhances collagen synthesis.[10] Epidermal growth factor is a 60 kd polypeptide that is normally found in salivary glands,



Immunohistochemical staining. A) (Patient 5) Staining for epidermal growth factor in patient with posttraumatic stenosis (original ×25). It is found distributed extensively in extracellular matrix of submucosal tissue. Minimal staining is seen within cytoplasm of fibroblasts or vascular structures. B) (Patient 4) Staining of tissue section from subject with fibrotic changes in submucosa and squamous metaplasia (original ×25). This section of subglottic stenosis is stained as control in absence of primary antisera. No immunostaining is seen. C) (Patient 5) Immunohistochemical staining for platelet-derived growth factor–AA from tracheal section (original ×40). Diffuse staining of extracellular matrix and within fibroblasts is present. Note lack of staining in endothelium of vessels and cytoplasmic staining in basal layer of epithelium. (Continued on next page.)



(Continued) D) (Patient 5) Prominent immunohistochemical staining for transforming growth factor–β2 within extracellular matrix (original ×25). Vasculature structures are not stained, and fibroblasts reveal minimal staining. E) (Patient 4) Prominent staining for platelet-derived growth factor–BB in tracheal section (original ×40). There was abundant granulation tissue present. This section shows mild matrix staining but significant staining of vasculature structures.

Brunner's glands, and the pancreas.[11] It acts on target cells via high-affinity cell surface receptors to stimulate protein tyrosine kinase, altering gene expression and inducing cell growth and differentiation while assisting wound and ulcer healing.[12,13] Basic FGF is a member of a family of heparin-binding growth factors capable of stimulating angiogenesis as well as augmenting proliferation of connective tissue cells.[14] Transforming growth factor–β is known to exist in different isoforms, and its biologic activities are extraordinarily complex.[15,16] It plays a major role in stimulating extracellular matrix formation by upregulating gene transcription of matrix proteins and protease inhibitors, while down-regulating collagenolytic enzymes.[17] Biologic effects include chemotactic activity on fibroblasts, monocytes, and neutrophils. Mitogenic activity is in part due to a paracrine induction of PDGF leading to fibroblast proliferation.[18] Subcutaneous injection of TGF-β predictably leads to rapid induction of scar tissue[19] and hence may serve to mediate a number of fibrotic clinical problems.[6]

Both TGF-β1 and TGF-β2 isoforms have been implicated in the pathogenesis of fibrosing diseases. In experimentally induced fibrosis, the expression of TGF-β1 mRNA correlates with excessive matrix deposition.[20] In humans with scarring chronic liver disease, elevated TGF-β1 mRNA in biopsies parallels mRNA production for type I procollagen and correlates directly with the serum levels of type III collagen amino terminal propeptide fragment.[21] Nonetheless, similar to our findings, studies have shown that TGF-β2 is found preferentially in fibrotic processes such as systemic sclerosis and proliferative vitreoretinopathy.[22,23] We found essentially no TGF-β1 staining, while TGF-β2 was prominent in all the idiopathic biopsy samples and four of the five post-intubation biopsy samples. That TGF-β2 was concomitantly expressed in the submucosa with PDGF suggests that PDGF and TGF-β2 may act in concert to promote fibroplasia and excessive extracellular matrix deposition, characteristic of SGS. Also, TGF-β2 and PDGF have been co-localized in studies of cutaneous fibrosis occurring in patients with eosinophilia-myalgia syndrome[24] and scleroderma.[25]

A significant correlation between the severity of fibrosis and the extent of either PDGF or TGF-β2 deposition could not be demonstrated in our analysis. This may be related to the relatively small numbers of patient specimens available for study. Also, wide variation in the extent of fibrosis observed among the biopsies further complicated our analysis. We attributed this wide variation in fibrosis mostly to differences in the timing of obtaining the sample during this dynamic disease process.

In summary, our study has demonstrated fibrogenic growth factors deposited within upper airway tissues of patients with SGS: in particular, TGF-β2 and PDGF-AA, and to a lesser extent, EGF and PDGF-BB. Some differences were noted between the idiopathic and the secondary forms of SGS, in particular with PDGF-BB and EGF staining. This may suggest that different pathogenic mechanisms are operative, although more studies are required to confirm this observation. Ideally, the control tissue for group 1 should derive from infants exposed to intubation but without clinical sequelae. This was not available to us, and thus, we utilized tissue from random postmortem cases.

Our experimental findings and an analysis of the literature lead us to propose that the presence of PDGF and TGF-β2 (and possibly EGF) may act in concert to produce this pathologic fibrotic process (SGS). The correlation of the growth factors with the presence of granulation tissue demonstrates their possible action as catalysts in the dynamic process of airway stenosis.

ACKNOWLEDGMENT—The authors thank Dr Stanley M. Shapshay for generously contributing specimens from patients with idiopathic subglottic stenosis for this study.

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Ann Otol Rhinol Laryngol 105:1996

TRACHEAL RESECTION WITH END-TO-END ANASTOMOSIS FOR ISOLATED POSTINTUBATION CERVICAL TRACHEA STENOSIS: LONG-TERM RESULTS

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A 20-year experience with end-to-end tracheal resection and anastomosis for isolated postintubation stenosis of the cervical trachea, in a consecutive series of 32 adult patients, has been reviewed. Surgical death was never encountered. The overall incidences for superficial wound infection, pneumonia, and inferior left laryngeal nerve paralysis were 6.2%, 3.1%, and 3.1%, respectively. One-, 3- and 5-year actuarial anastomosis success rates were 96.7%, 93.3%, and 93.3%, respectively. Successful revision tracheal end-to-end anastomosis was performed once, resulting in an overall 96.9% success rate in our series. None of the following variables — sex, age, cause for intubation, intubation type (laryngotracheal and/or tracheotomy) and duration, delay from initial injury, presence of an open stoma, number of tracheal rings resected, and type of sutures used — were statistically related to the anastomosis success rate or the incidence of complications.

KEY WORDS — anastomosis, intubation, stenosis, trachea.

INTRODUCTION

Prolonged ventilation and advances in critical care medicine, cardiopulmonary surgery, and neurosurgery have resulted in an increased number of intubation-related laryngotracheal injuries.[1,2] Within the trachea, trauma at the tracheotomy site or the cuff or the tip of the ventilation tube might result in granulation tissue formation.[3] Keane et al,[4] in a review article on complications of intubation, noted that the same factors that influence the incidence and severity of laryngeal complications (size of the tube, duration of intubation, intubation trauma, movement of the tube within the trachea, chemical composition of the cuff and tube, and cuff pressure in excess of 30 mm Hg) also act within the trachea. Following the onset of granuloma at the injury site, the reparative process may lead to the formation of fibrous tissue with a compromised airway.

Curative options for isolated postintubation stenosis of the cervical trachea varied from repeated dilations, laser vaporization, excision of stenosis, and stenting with various interposition grafts to tracheal resection with primary anastomosis.[2,5-7] Various reports underscore the value of tracheal resection with end-to-end anastomosis for postintubation cervical trachea stenosis.[5,8-10] However, to our knowledge, no report has specifically analyzed the long-term results of tracheal end-to-end anastomosis for isolated postintubation stenosis of the cervical trachea.

This retrospective study was designed in an attempt to assess the long-term results of circumferential resection with end-to-end tracheal anastomosis for isolated postintubation stenosis of the cervical trachea. Causes for stenosis are presented. This study focuses on anastomosis success rate, morbidity, and postoperative course. The cervical trachea end-to-end anastomosis success rate was analyzed with the Kaplan-Meier[11] life-table method. Furthermore, the following independent variables — sex, age, cause for intubation, intubation type (laryngotracheal and/or tracheotomy) and duration, delay from initial injury, presence of an open stoma, number of tracheal rings resected, and type of sutures used — were tested for potential correlation with the incidence of complications and the success rate of end-to-end cervical trachea anastomosis.

MATERIALS AND METHODS

From 1970 to 1990, 263 adults patients with intubation-related laryngotracheal injury were consecutively managed at our department. A retrospective analysis of the medical charts revealed that a circumferential resection with end-to-end anastomosis for an isolated postintubation stenosis of the cervical trachea was performed in 12.1% (32/263) of cases. The patient population comprised 13 women and 19 men with ages ranging from 15 to 71 years (mean 37 years). Patients who underwent cricotracheal anastomosis, required a sternotomy for resection of a cervicomediastinal tracheal stenosis, and/or presented with associated intubation-related laryngeal injury,

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INTUBATION PARAMETERS (CAUSE, DURATION, DELAY) AND TREATMENT PERFORMED PRIOR TO TRACHEAL ANASTOMOSIS

Cause of intubation		
Coma (barbiturate)	10	(31.2%)
Coma (trauma)	4	(12.5%)
Coma (trauma and neurosurgery)	2	(6.3%)
Polytraumatic surgery	6	(18.8%)
General surgery	3	(9.4%)
Heart surgery	2	(6.3%)
Tetanus	2	(6.3%)
Quincke's edema	1	(3.1%)
Guillain-Barré syndrome	1	(3.1%)
Vascular cerebral injury	1	(3.1%)
Intubation duration (d)		
Endotracheal intubation		
Range	2-30	
Mean	8	
Tracheotomy		
Range	2-960	
Mean	80	
Delay from initial intubation (mo)		
Range	2-60	
Mean	11	
Prior treatments		
Multiple dilations	20	(62.5%)
Oral steroids	10	(31.3%)
Laser vaporization	2	(6.3%)
Montgomery T-Tube insertion	1	(3.1%)
No prior treatment	12	(37.5%)

were excluded from the present study.

The cause and duration for intubation, the delay from initial intubation, and the prior treatment performed are presented in the Table. Laryngotracheal intubation with subsequent tracheotomy was noted in 12 patients. Isolated laryngotracheal intubation and isolated tracheotomy were noted in 15 and 5 patients, respectively.

None of the patients within this series presented with preoperative aspiration and/or tracheoesophageal fistula. Six patients had an open stoma. Neurologic impairment from central nervous system damage included hemiplegia and Wernicke (receptive) aphasia in 2 patients and 1 patient, respectively. One patient presented with unilateral inferior laryngeal nerve paralysis.

The preoperative workup included indirect laryngoscopy, tracheobronchoscopy, and laryngotracheal tomography or computed tomography. Tracheal stenosis was staged as stage 2 (scarring with some inflammatory process) and stage 3 (complete mature stenosis) in 10 and 22 patients, respectively. The tracheal stenosis was complete in 2 patients. A 50% to 90% reduction in the internal diameter was noted in 23 patients. In the remaining 7 patients the internal tracheal diameter was reduced by more than 90%.

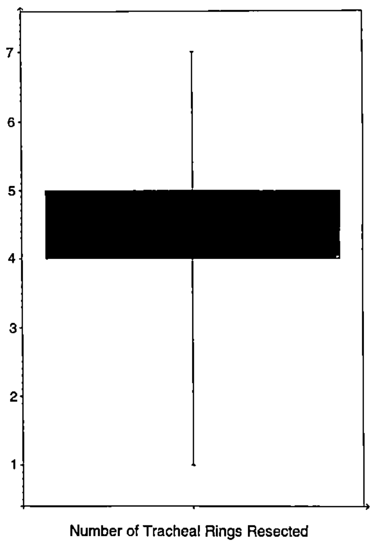

Fig 1. Number of tracheal rings resected (box-plot diagram). Data are from operative file and pathologist report.

Five surgeons were in charge of the patients. Twenty-five patients (78%) were operated on by one of us (H.L.). Anesthesia management with the use of high-frequency jet ventilation, similar to that of other reports,[6,8] will not be described. Once the neck was maximally extended and the subplatysma flap was elevated, a blunt dissection between the sternohyoid muscles and the sternothyroid-thyrohyoid muscles was performed bilaterally. The strap muscles were then retracted laterally and the trachea was exposed in the midline. The thyroid isthmus was transected. Anterior and lateral pretracheal cervical and mediastinal blunt dissection with mobilization down to the carina was always performed. The stenotic area was identified and resected with a dissection close to the tracheal wall. No attempt was made at identifying the inferior laryngeal nerves. The circumferential segment of trachea resected varied from 1 to 7 tracheal rings (4 or 5 rings on average, Fig 1). The preexisting stoma was resected with the stenotic tracheal segment (3 patients) or just closed in a simple manner (3 patients). The upper tracheal ring used for tracheal anastomosis completion was the 3rd on average (range, 1st through 9th). The lower tracheal ring used for tracheal anastomosis completion was the 9th on average (range, 4th through 14th). The tracheal anastomosis was performed with polyglactin (Vicryl 30) sutures and polyester fiber (Tevdek 30) sutures in 20 and 12 patients, respectively. Sutures were always set submucosally through healthy mucosa without granuloma or infection. Neck flexion was always performed and the sutures were placed by starting from the posterior midline and moving laterally on both sides.[3,6,8] The anastomosis was then completed ante-

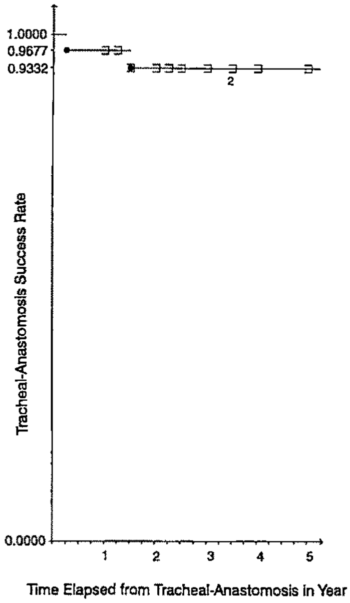

Fig 2. Actuarial anastomotic success rate (Kaplan-Meier actuarial method). Discs — patients presenting restenosis after tracheal anastomosis; open-ended squares — patients without restenosis who were either dead or lost to follow-up at time of latest follow-up.

riorly. An infrahyoid laryngeal release maneuver was used in 1 patient. No other maneuvers to reduce the tension at the level of the anastomosis line were used in this series. A suction tube was inserted into the wound. The strap muscles were closed on the midline. The skin incision was closed in two layers. Closed suction drainage was used for 48 hours.

A control tracheoscopy under general anesthesia was performed by the end of the first postoperative month. Morbidity and tracheal anastomosis success rate were assessed clinically. The tracheal anastomosis was considered as successful if the patient was asymptomatic with office flexible tracheoscopy demonstrating a patent airway.

A minimum 2-, 3-, and 5-year follow-up was available in 100%, 87.5% (28/32), and 78.1% (25/32) of patients, respectively. Three patients living outside France were lost to follow-up by the end of the second postoperative year. A monitoring program was applied for all patients from the date of treatment until death for those who died. An IBM PC computer with PCSM (Programme Conversationnel de Statistique pour les Sciences et le Marketing, Deltasoft Company, Meynan, France) software was used for storing and calculating statistical data. The tracheal anastomosis success rate was analyzed by the Kaplan-Meier life table method.[11] The parametric χ^2 test and the nonparametric Mann-Whitney U test were also used in order to assess whether or not the following independent factors — sex, age, cause for intubation, intubation type (laryngotracheal and/or tracheotomy) and duration, delay from initial injury, presence of an open stoma, number of tracheal rings resected, and type of sutures used — influenced the incidence of complications or the tracheal anastomosis success rate.

RESULTS

At the termination of the procedure in the operating room, all patients could ventilate freely. Reintubation was never performed.

Postoperative complications included superficial wound infection, left inferior laryngeal nerve paralysis, pneumonia, and keloid scar in 2, 1, 1, and 2 patients, respectively. Control tracheoscopy by the end of the first postoperative month revealed a granuloma in 8 patients (25%). Seven of the 8 granulomas were successfully managed with endoscopic excision at the time of control tracheoscopy and/or oral steroid treatment. None of the variables under analysis were statistically related to the type or incidence of postoperative complications or granuloma formation. Oral intake was achieved in all patients by the second postoperative day. Postoperative dysphagia was not encountered.

Figure 2 presents the actuarial anastomotic success rate for the entire group of patients. One-, 3-, and 5-year actuarial anastomotic success rates were 96.7%, 93.3%, and 93.3%, respectively. Two patients in our series had a restenosis. None of the variables under analysis were statistically related to the incidence for restenosis. One patient developed immediate postoperative extensive granulation tissue at the anastomosis site that required permanent placement of a Montgomery T-Tube. In the second patient, recurrence of dyspnea by the second postoperative year led to a successful revision tracheal resection with end-to-end tracheal anastomosis. An overall 96.9% (31/32) success rate was therefore achieved within this series.

DISCUSSION

In 1992, Anand et al[12] noted that despite improvements in intubation management, the incidence of tracheal stenosis was 0.6% to 21% following tracheotomy and 6% to 21% after intubation. Endotracheal intubation followed by a subsequent tracheotomy has also been related to an increase in tracheal stenosis incidence and severity.[2,13]

Treatment options for isolated postintubation stenosis of the cervical trachea have included an endoscopic approach with repeated dilations and laser vaporization, excision of the stenosis with various interposition grafts and stenting, and tracheal resection with primary anastomosis.[2,5-7] The success rate for the endoscopic dilation and laser vaporization procedures was extremely reduced if the stenosis was circumferential, greater than 1 cm in vertical length, and without cartilaginous support.[7] In our series, prior unsuccessful dilation and/or laser vaporization was noted in 68.7% of cases. Excision of stenosis with interposition techniques and stenting is mainly advocated for complex lesions involving the larynx and trachea.[2,6] Interposition techniques were not used in our series, which represents a selected population without associated intubation-related laryngeal injury. Circumferential tracheal resection with end-to-end anastomosis remains the most widely advocated option for postintubation stenosis of the cervical trachea, as this procedure yields a 67.6% to 94.7% success rate.[5,8-10,14,15] Grillo,[10] in 1979, in the largest reported series, based on 216 patients, reported an overall 93% success rate with a 2% operative mortality rate.

Experimental data underscored that the primary requirement for the success of tracheal end-to-end anastomosis was the completion of a tension-free anastomosis line.[12] According to Montgomery,[16] tension should not exceed 1,000 g at the suture line. Cantrell and Folse,[17] in an animal study, reported that no stricture formation occurred if there was less than 1,700 g of tension on the suture line. Numerous techniques (heavier sutures and sutures placed between the rings superior and inferior to the anastomosis line, chin-to-chest suture), as well as release procedures (suprahyoid laryngeal release, intercartilaginous incisions, inferior constrictor release, release of the inferior pulmonary ligament, transection and reimplantation of the left main stem bronchus), have been advocated for gaining additional tracheal length at the time of tracheal anastomosis.[1,3,5,6,16,18] Peskind et al[6] recently advocated the routine use of a supralaryngeal release procedure (combined infrahyoid and suprahyoid) to decrease tension at the level of the anastomosis suture line. However, in their series, cinefluoroscopy documented that laryngeal release procedures fixed the larynx in a low position in the neck and prevented elevation during swallowing, thus producing postoperative dysphagia in all patients.[6] Biller and Munier[5] also reported that the combined infrahyoid and inferior constrictor release resulted in a 20% incidence of prolonged postoperative dysphagia. On the other hand, various reports have underscored that a tracheal defect of 2 cm or less can be successfully managed by primary anastomosis alone, without release techniques.[16,19,20] In our series, resection varied from 1 to 7 tracheal rings, resulting in a 1- to 3.5-cm length of cervical trachea resection, as there are approximately 2 tracheal rings per centimeter.[21] A laryngeal release procedure was used only once, and a 93.3% 5-year actuarial anastomotic success rate was achieved (Fig 2). Revision end-to-end tracheal resection was successfully performed once in our series, allowing for an overall 96.9% success rate. Our results suggested, therefore, that a tracheal defect of 3.5 cm or less can be successfully managed by primary anastomosis alone, without release techniques. Such data are in agreement with the cadaver study of Laccourreye and Bessede,[22] who noted that after resection of 3 to 6 tracheal rings, the intertracheal ring ligament elasticity allowed for tracheal end-to-end anastomosis without any adjunctive measures other than mediastinal tracheal dissection and wide blunt cervical dissection between the sternohyoid and thyrohyoid-sternothyroid muscles. Besides the completion of a tension-free anastomosis line, numerous reports also underscore the need to perform the sutures through a healthy mucosa.[14,23] In our study, the sutures were always set through a healthy mucosa without granuloma and/or infection. Furthermore, none of the variables under analysis in our study — sex, age, cause for intubation, intubation type (laryngotracheal and/or tracheotomy) and duration, delay from initial injury, presence of a stoma, number of tracheal rings resected, and type of sutures used — statistically influenced the success of end-to-end cervical trachea anastomosis.

The timing of extubation after tracheal end-to-end anastomosis is controversial. Various authors advocate leaving the patient intubated for 24 to 48 hours to achieve an airtight suture before exposure to positive intratracheal pressure.[5,6,18] In our opinion, maintaining an endotracheal tube presents a risk of injury to the anastomosis or a different segment of the trachea. Therefore, in our department, extubation was always performed in the operating room. As in the report of Har-El et al,[8] immediate extubation was always successfully performed in our series. Potential complications after tracheal end-to-end anastomosis include airway obstruction, infection of the operated region and/or tracheal wall, innominate artery rupture, and dysphagia.[6,24] Couraud et al[24] reported a 3.3% incidence for perioperative death after end-to-end tracheal resection anastomosis among 122 patients with tumoral or iatrogenic disease. None of these complications were noted in our series. Postoperative complications included superficial wound infection, left inferior laryngeal nerve paralysis, and pneumonia in 2, 1, and 1 patients, respectively. The systematic completion of a tracheoscopy by the first postoperative month also revealed granuloma in 25% of patients. Such data are in agreement with the report of Grillo,[10] who noted granulations after tracheal anastomosis in 34 of 193 patients. In our series, none of the variables under analysis were statistically related to the incidence of postoperative complications or tracheal granuloma.

In conclusion, this report reemphasizes that whenever possible, primary resection with end-to-end anastomosis via a cervical approach is a relatively safe and reliable procedure for isolated postintubation stenosis of the cervical trachea. Tracheal resection with end-to-end anastomosis obtains wound healing by primary intention with a low complication rate and a high success rate. In patients in whom 1 to 7 tracheal rings are resected, our study suggested that end-to-end tracheal anastomosis with mediastinal tracheal dissection and blunt dissection between the deep and superficial infrahyoid muscles 1) does not require the systematic use of adjunctive measures to release tension at the level of the suture line and 2) results in long-term airway patency.

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Ann Otol Rhinol Laryngol 105:1996

CRANIAL NERVE AND HEARING FUNCTION AFTER COMBINED-APPROACH SURGERY FOR GLOMUS JUGULARE TUMORS

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In this retrospective study, oncologic and functional results of 46 patients treated for glomus jugulare tumor are reported. The standard surgical approach was the combined transmastoid-transcervical approach, modified according to the individual tumor growth, and eventually combined with a transtemporal or a suboccipital approach. Complete tumor removal resulted in a cure rate of 90%. New-onset cranial nerve palsies developed in less than 22% of patients. In 54% of cases it was possible to retain middle ear function. From a total of 12 patients with incomplete tumor removal and postoperative irradiation, progressive tumor growth was noted in 4 patients, and was controlled by salvage irradiation or surgery. Radical tumor removal by ablative surgery can be modified by efforts to reduce mutilating resections. In their place, individually tailored and combined multidirectional surgical approaches may allow total tumor removal with lower morbidity.

KEY WORDS — glomus jugulare tumor, surgery.

Although the management of glomus jugulare tumors has been a subject of controversy since the initial description by Rosenwasser,[1] the only curative treatment is undoubtedly radical surgery.[2,3] In large patient series, cure rates of 86% to 100% after complete surgical removal have been reported.[4] Still, surgery for glomus jugulare tumors remains difficult and challenging. Giant tumors with invasion of the internal carotid artery (ICA), the clivus, and the pontomedullary region increase the surgical risk, and their removal sometimes produces new morbidity, nonexistent preoperatively and caused by surgical injury to important adjacent structures. Woods et al[4] reported that the vagus and glossopharyngeal nerves were anatomically preserved in 49% and 32% of patients, respectively. The facial nerve was preserved in 80% of patients. Poe et al[3] stated that more than half of the patients presented with cranial nerve deficits, and after operation 84.5% had some cranial nerve deficit. Conductive hearing loss is the consequence of the infratemporal fossa approach described by Fisch and Mattox,[5] even in patients with preoperatively normal hearing. In their controlled series of 36 patients, followed up for 6 to 10 years, from a total of more than 240 operated tumors, the functions of the 9th and 10th cranial nerves deteriorated in 33% of cases postoperatively (U. Fisch, personal communication, 1995).

The aim of this study was to reassess our 20-year experience with the surgery of glomus jugulare tumors with particular reference to the postoperative morbidity in terms of cranial nerve involvement and hearing preservation. Total tumor removal remained the ultimate goal, but the individual planning of surgical access was oriented toward the least destructive procedure.

MATERIAL AND METHODS

Patient Population. Twenty-seven patients with glomus jugulare tumors class C according to Fisch and Mattox,[5] and 19 patients with class D tumors, treated at the Department of Otorhinolaryngology in Erlangen between 1973 and December 1993, were submitted to the present retrospective study (Fig 1). Data relating to presenting symptoms, physical, radiologic, and audiologic findings, cranial nerve status, and treatment modalities were analyzed and tabulated. The evaluation of every parameter was not possible in all cases. The routine follow-up consisted of yearly clinical examination, and computed tomography or magnetic resonance imaging (MRI) in the first 3 years after the treatment and later in 3- to 5-year

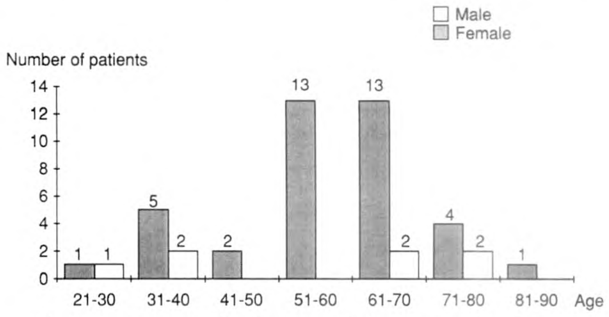

Fig 1. Age and sex distributions of 46 patients with glomus jugulare tumors (tumor sizes C and D).

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TABLE 1. TREATMENT MODALITIES IN MANAGEMENT OF GLOMUS JUGULARE TUMORS

Treatment Modality	Tumor Sizes (Fisch Classification) C	D	% of Patients (N = 46)
Complete tumor removal with no irradiation	16	7	50
Incomplete tumor removal followed by radiotherapy	7	9	35
Radiotherapy	4	3	15

intervals. The present study was additionally based on a current clinical evaluation in 26 patients and a detailed questionnaire in 6 patients, while 7 patients had died of causes unrelated to glomus tumor. Seven patients were lost to long-term follow-up, but some aspects could still be evaluated. The average follow-up interval was 89 months, with a range from 8 to 248 months.

Left-sided tumors were more frequent, with a left-right ratio of 30 to 16. Two patients had an additional carotid body paraganglioma. Preoperative catecholamine screens were not routinely performed, but no tumor in this series showed clinical signs of neuropeptide or vasoactive amine secretion.

Surgical Treatment. Twenty-three patients were treated surgically with complete removal of the neoplasm as evidenced by the surgical microscope (Table 1). There were 20 women and 3 men. Ages ranged at the time of presentation or diagnosis from 31 to 73, with a median age of 56. The average duration of symptoms at the time of referral was 4.6 years (range 1 to 17).

In a further 16 patients the tumor was resected subtotally and these patients received postoperative radiotherapy. There were 13 women and 3 men. Ages ranged from 24 to 74, with a median age of 56. The average duration of symptoms was 4.4 years (range 1 to 21). This group of patients was in every respect comparable with the patients treated only surgically, with the exception that more tumors showed intracranial extension (Table 1). The reasons for incomplete tumor resection were as follows: refusal of a planned second-stage surgery by the patient; interruption of the surgery because of severe hemorrhage, early in the series; and the fact that in some patients with a limited life expectancy (over 65 years old, low physical performance) and intact lower cranial nerves, microscopically small tumor remnants were deliberately left at the jugular foramen or on the ICA.

The standard surgical approach was the *transmastoid-transcervical approach*, which was modified according to the individual tumor growth, and eventually combined with a transtemporal exposure of the petrous bone or with a lateral suboccipital approach (Table 2). Limited approaches like the transcanal and the transmastoid approaches were used as the first operation in patients who later refused further surgery.

TABLE 2. SURGICAL APPROACHES

Approach	Tumor Sizes (Fisch Classification) C	D	% of Patients (N = 39)
Transcanal	3	0	8
Transmastoid	4	3	18
Transmastoid-transcervical	15	5	51
Transmastoid-transcervical followed by neurosurgery*	1	8	23

*Including infratemporal fossa approach and transtemporal approach.

The combined transmastoid-transcervical approach was an extension of the retroauricular transmastoid approach and permitted not only the exposure and the control of structures lying at the jugular foramen or in the posterior fossa, but also a control of the ICA in its petrous portion and in the upper neck. The technique basically corresponds to that described by Glasscock et al.[6] The facial recess was opened from posterior and enlarged inferiorly behind the annulus with sacrifice of the chorda tympani. The facial nerve was skeletonized in the mastoid portion and temporarily or permanently transposed. In the second phase of this operation the lower cranial nerves and the ICA were exposed from the neck into the petrous bone. Proximally, the sigmoid sinus was ligated or occluded with extraluminal packing of oxidized cellulose. The internal jugular vein was ligated in the neck, and the jugular bulb, containing the tumor, was resected with the dura of the posterior fossa respected as much as possible. Meticulous repair of any dural defect and obliteration of the mastoid cavity with free muscle-periosteum grafts or with pedicled muscle terminated the procedure. Preservation of the middle ear structures was always attempted when serviceable hearing existed preoperatively. Reconstruction of the tympanic membrane and the ossicles was accomplished at the end of the procedure or in a second operation. If the eustachian tube was severed by tumor or surgery, a ventilation tube was inserted into the tympanic membrane. This was usually performed 3 months after surgery to minimize the risk of cerebrospinal fluid (CSF) otorrhea.

In patients with large intradural tumors a *combined otoneurosurgical procedure* was performed (Fig 2). The posterior fossa dura was opened and resected according to the tumor extent in the posterior fossa. An exposure of the ICA by the *combined trans-*

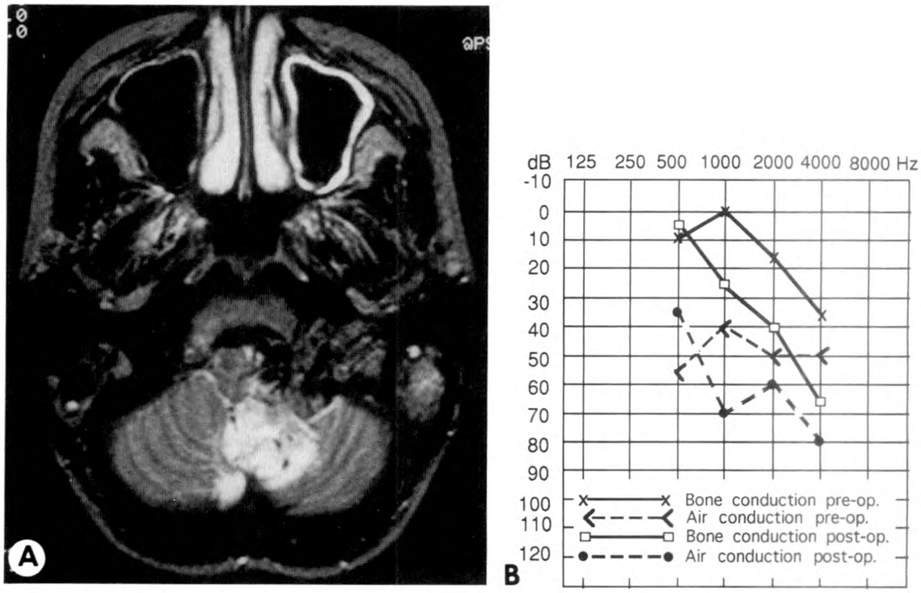

Fig 2. Thirty-year-old patient with 2-year history of headache and vagus nerve palsy demonstrating giant paraganglioma, C3Di2. Total tumor removal was accomplished by two-stage, combined transmastoid-transcervical-transtemporal approach, producing no further cranial nerve deficit. A) Preoperative T2-weighted magnetic resonance image. B) Hearing before and 14 months after surgery.

temporal and transmastoid-transcervical approach was used for tumors reaching in front of the carotid knee to its horizontal portion without cochlea involvement.[7,8] The function of the middle ear could thus be preserved. Such wide superior and inferior exposure also reduced the need for facial nerve manipulation. In only 2 cases, the *infratemporal fossa approach* of Fisch and Mattox,[5] with obliteration of middle ear spaces, became necessary in this series. Both patients had a class C3De1 tumor and were preoperatively deaf because of extensive destruction of the labyrinth.

The surgery was staged in 11 of 16 patients with a class D tumor because of a large intradural tumor extension or because of the long duration of the operation. In comparison, only 7 of 23 patients with class C tumors had staged surgery.

Eighteen tumors were embolized 1 to 3 days preoperatively, and 1 tumor was embolized prior to irradiation. In the same session a preoperative balloon test occlusion of the ICA in combination with single-photon emission computed tomography (SPECT) measurement of the cerebral blood flow was performed in 2 patients with the tumor encasing, but not narrowing, the ICA, as seen on MRI.[9] Both patients tolerated the test well clinically. One showed a decrease of cerebral blood perfusion in the SPECT; however, surgery was uneventful, and the tumor could be separated from the ICA.

TABLE 3. CURE RATES AFTER DIFFERENT TREATMENTS OF GLOMUS JUGULARE TUMORS

Primary Treatment Modality	*Tumor Sizes (Fisch Classification)*		*% With Tumor Control*
	C	*D*	
Total removal (N = 20)*	13/14	5/6	90
Incomplete removal followed by radiotherapy (N = 12)*	4/5	4/7	67†
Radiotherapy (N = 7)	4/4	3/3	100†

*Seven patients were lost to follow-up.

†Patients free of symptoms, tumor size constant on magnetic resonance imaging.

Radiotherapy. Seven patients had only radiotherapy; this was because of bad physical performance in 5 patients, an unrelated malignant disease in 1 patient, and refusal of surgery by 1 patient (Table 1). There were 5 women and 2 men. Ages ranged at the time of presentation or diagnosis from 57 to 84, with a median age of 70. The average duration of symptoms at the time of referral was 3.8 years (range 1 to 11). Irradiation was delivered with 6-MeV photons using multiple field techniques including wedge filters. The total dose ranged from 45 to 60 Gy, in recent years figuring 45 Gy in 1.8-Gy daily fractions over 5 weeks.

RESULTS

Results are shown in Tables 3-5 and Fig 3. Out of a consecutive series of 46 patients with large glomus jugulare tumors (class C and D), 39 could be submitted to a comparison of different treatment modalities (Table 3). Seven patients moved away and were lost to long-term follow-up.

In the group of 20 patients with *total tumor removal* and an average follow-up interval of 70 months (8 to 248 months), 2 residual tumors presented, 1 in the class C group and 1 in the class D group. The time interval between surgery and the detection of tumor regrowth was 3 years and 4 years, respectively. One patient received two courses of salvage irradiation (50 Gy in 1986 and 12 Gy in 1989) and additional

TABLE 4. PATIENTS WITH PREOPERATIVE AND PERMANENT POSTOPERATIVE CRANIAL NERVE PALSIES

	Total Tumor Removal (Fisch Classification)		Subtotal Tumor Removal Followed by Radiotherapy (Fisch Classification)	
Nerves	C (N = 16)	D (N = 7)	C (N = 7)	D (N = 9)
VII (House II-VI)	2, 4	2, 5	1, 2	1, 4
IX	1, 3	3, 5	1, 1	1, 2
X	1, 4	6, 7	1, 2	3, 4
XI	0, 4	0, 1	0, 0	1, 1
XII	0, 2	0, 3	0, 0	3, 5

Data are preoperative number followed by postoperative number.

radical surgery in 1992, and is at present doing well without clinical or radiologic signs of tumor presence. The other presented recently in a very bad condition with a large intradural tumor. A ventriculoatrial shunt was placed and she received a course of palliative irradiation.

Of a total of 12 patients with *incomplete tumor removal and postoperative irradiation* (total dose range 45 to 60 Gy), progressive tumor growth was noted in 4 patients, 3 to 6 years after the treatment. Follow-up in this group averaged 80 months (9 to 194). All 4 patients with tumor progression had a second course of radiotherapy (40 to 45 Gy), and 1 of them had revision surgery. Meanwhile, 2 patients are well with tumors showing no progression, and 2 died of causes unrelated to the paraganglioma.

In the pure *radiotherapy* group (7 patients) there were no tumor progressions as confirmed by serial MRI. The follow-up of 3 patients was only 2 years, another 3 patients died from diseases unrelated to the tumor within 2 years, and 1 patient with a follow-up of 5 years is doing well.

The clinical status of the cranial nerves was analyzed in all 39 surgical cases (Table 4). Permanent new-onset facial nerve palsy developed in 3 patients with a class C tumor and in 6 patients with a D tumor. Of those, only 2 (C tumors) and 3 (D tumors) facial paralyses were House grade V or VI. Two of them were caused by nerve mobilization during surgery, and 3 by resection of the tumor-infiltrated nerve.

TABLE 5. POSTOPERATIVE COMPLICATIONS

	Tumor Sizes (Fisch Classification)		% of Patients
	C	D	(N = 39)
Meningitis		3	8
Cerebrospinal fluid leak	1	5	15
Labyrinthitis		1	3
Nerve VII paralysis (House V-VI)	2	3	13
Nerve IX paralysis	2	3	13
Nerve X paralysis	4	2	15
Nerve XI paralysis	4	1	13
Nerve XII paralysis	2	5	18
Perioperative mortality	2		5

Lower cranial nerve functions in the group of patients with subtotal removal, when compared with the group with total tumor removal, showed more favorable results in the first group. All of the patients with deficits of the lower cranial nerves were successfully physically rehabilitated. The nutrition was controlled by intravenous alimentation until nasogastric tube feeding was possible. It was unnecessary to perform either a tracheotomy or a gastrostomy for their functional rehabilitation. Thyroplasty with medialization of the true vocal cord[10] was performed in 1 patient for speech rehabilitation.

In irradiation-only cases there were no new-onset cranial nerve palsies.

Hearing results were tabulated comparing the pure tone audiogram (PTA) curves before surgery and at the last presentation in 30 surgical patients (Fig 3). The audition deteriorated in 7 patients after the surgery, remained unchanged in 20 patients, and improved in 3 patients.

In irradiation-only cases, 3 patients were already deaf at the first presentation. The hearing of 2 patients remained unchanged after the treatment (45 Gy, 46 Gy), and 2 patients lost their hearing completely (64 Gy, 54 Gy).

Pure tone thresholds (Average of 0.5,1,2,4 kHz)

☐ = class C
▣ = class D

pre - OP \ post - OP	0 - 30 dB	31 - 60 dB	61 - 90 dB	> 90 dB
0 - 30 dB	☐☐☐ ▣▣▣		☐	
31 - 60 dB	▣	☐☐☐ ▣▣	☐ ▣▣	☐☐
61 - 90 dB		▣		▣
> 90 dB			☐	☐☐☐☐☐ ▣▣▣▣

Fig 3. Hearing results.

The perioperative mortality rate was 5% (Table 5): 2 patients, ages 62 and 58, died 7 and 10 days, respectively, after the operation. Autopsy revealed that in 1 patient a cerebral apoplexy had occurred, and the other died of brain edema due to cerebral ischemia caused by the supine and distorted position of the head in the presence of a malformation of the vertebral arteries.

DISCUSSION

Impact of Therapy on Tumor Status. Improvements in the surgery of glomus jugulare tumors during the last decades contradict the earlier argument against surgery, namely, a high percentage of residual tumors and significant postoperative morbidity.[5,6,11,12] Woods et al[4] reported The Otology Group (Nashville, Tenn) experience, also detailed by Poe et al,[3] with 43 consecutive patients and observed no tumor recurrences. The duration of follow-up ranged from 2 to 19 years, with a median of 5 years. Anand et al[13] observed no recurrence or persistence of tumor, clinically or radiographically, 4.5 years after surgery in 16 of 20 patients with large tumors having intracranial extension.

In our series of glomus jugulare tumors, 90% of patients were tumor-free after total tumor removal, as confirmed by serial imaging and clinical examinations, and an average follow-up of 70 months. The overall results support our preference for surgery, which offers a definitive treatment and the best long-term cure rates. Radiotherapists claim a local tumor control rate of about 90% in irradiation patients.[14,15] In our patients treated by incomplete surgical removal and full-dose irradiation, a significantly poorer outcome was seen in comparison to those treated only surgically. Four of 12 patients experienced progressive tumor growth after a primary treatment consisting of subtotal surgery and subsequent radiotherapy. The follow-up averaged 80 months in this group. However, after salvage therapy all 4 patients were doing well. Nevertheless, salvage irradiation was not able to prevent tumor from growing in 2 patients, and they received revision surgery.

The experience with combined surgical and irradiation treatment was gathered early in the series with patients that refused the planned second-stage surgery, and was afterward employed in some surgical candidates with few preoperative symptoms but limited life expectancy. The surgery was always started with the goal of total tumor removal, and the decision to leave a tumor remnant was made intraoperatively and was the result of balancing the predictable complications from further radical ablation (lower cranial nerve impairment, involvement of the ICA and the vertebrobasilar system, etc) against the biologic age of the patient. In those patients with poor collateral cerebral circulation, as evidenced by angiography and balloon test occlusion of the ICA with SPECT, radical dissection of the tumor from the artery was not carried out.

Our group of 7 patients treated by radiotherapy alone was far too small, and the follow-up too short, to allow any significant conclusion.

Hearing and Cranial Nerve Function. The benefit of radical tumor removal by extensive surgery should be followed by efforts to reduce the functional impairments necessary to achieve complete resection. The provision of an adequate and safe exposure of the tumor and adjacent neurovascular structures is of utmost importance. For the removal of class C and D tumors, Fisch and Mattox[5] advocate the infratemporal fossa approach, which routinely includes the removal and blind sac closure of the external auditory canal, and the exenteration of pneumatic middle ear spaces with subsequent obliteration of the cavity, producing a maximal conductive hearing loss. Jackson et al,[2] in their series of class C tumors, reported that 19% of patients were operated on by the canal wall up technique, 25% by the canal wall down technique, and 56% by the infratemporal fossa approach. Even in class D1 tumors, 18% were operated on by the canal wall up technique, 6% by the canal wall down technique, and 76% by the infratemporal fossa approach.

We have, however, come to the conclusion that individually tailored and combined, multidirectional surgical approaches — eg, transmastoid-transcervical in combination with a transtemporal or suboccipital exposure — enable total tumor removal with low morbidity. Patel et al[16] recently published an excellent review on combined approaches for resection of extensive glomus jugulare tumors and added 12 of their own patients. Subtemporal-infratemporal, retrosigmoid, and/or extreme lateral transcondylar approaches were added to the transtemporal-infratemporal approach. They concluded that the improved exposure provided early control of the petrous ICA, reduced the necessity for facial nerve mobilization, and facilitated tumor removal from the clivus, cavernous sinus, posterior fossa, and foramen magnum, allowing a single-stage resection in 8 patients. However, they further stated that the permanent conductive hearing loss is acceptable morbidity, and that efforts to preserve hearing are not suitable, specifically in tumors that are anteromedial to the ICA or extend intracranially. Even in large tumors, we did not routinely remove the posterior auditory canal wall, nor did we obliterate the middle ear, but tried to save a functioning ventilated middle ear. In order to realize this principle, an extended transmastoid-transtympanic approach was utilized, removing and reconstructing the posterior canal wall as far as necessary, displacing the facial nerve temporarily if necessary, and dissecting the jugular bulb and the vertical portion of the ICA from two directions: from below and from above via the middle fossa approach when the tumor had embedded the carotid knee.[7,8] The cochlear and labyrinthine structures were thus preserved if still functioning. Tumors causing encasement with displacement of the ICA could frequently be dissected from the artery. The tunica media resists the tumor infiltration for a long period of time. No tumor in this series had preoperatively shown encasement with narrowing of the ICA on MRI or on the angiographic studies. Permanent anterior transposition of the facial nerve was seldom used; a temporary and partial transposition of the nerve beginning from the tympanic portion was preferred. Dissection above and below the facial nerve, through the wide transtemporal and transmastoid-transcervical exposure, resulted in the fact that in some cases the nerve was only skeletonized, but not mobilized. Reconstruction of the eardrum and the ossicles was attempted at the end of the procedure or at a second stage. By using the aforementioned technique, the deterioration rate of cranial nerve function in surgery-only cases could be kept low in comparison with the data from the literature[3-5] — for the facial nerve 13%, the glossopharyngeal nerve 17%, and the vagus nerve 17%.

One of the main arguments in favor of blind sac closure of the external auditory canal and obliteration of the middle ear spaces is the prevention of CSF leak.[5] Woods et al[4] observed a postoperative CSF leak in 10 of 71 patients, and Anand et al,[13] in 1 of 20 patients. Despite preservation of the middle ear and the external auditory canal, a CSF leak occurred in only 6 of 39 tumor operations in our series. Surgical revision resulted in control of the CSF leak in all cases. We accept a somewhat higher percentage of CSF leaks, to be controlled without major problems, when the end result is the preservation of hearing.

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Ann Otol Rhinol Laryngol 105:1996

INTERSTITIAL IODINE 125 IN ADVANCED RECURRENT SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK WITH FOLLOW-UP EVALUATION OF CAROTID ARTERY BY ULTRASOUND

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We retrospectively reviewed our experience with 24 patients in the treatment of advanced recurrent squamous cell carcinoma of the head and neck, using salvage surgery and intraoperative iodine 125 (^{125}I) implantation. Surgical complications and survival results were compiled and compared with those of other studies. The long-term effect of ^{125}I on the carotid artery was evaluated by ultrasound. We had a major complication rate of 21% and an overall complication rate of 50%. Our 2-year overall and determinate survivals were 29% and 50%, respectively. Within the survival group, carotid ultrasounds were obtained to evaluate the long-term effect of ^{125}I. All ultrasounds obtained at least 1 year from the time of treatment showed minimal or no change from the contralateral side. We conclude that intraoperative ^{125}I and salvage surgery are an acceptable treatment for recurrent squamous cell carcinoma with minimal complication and effect on the carotid artery.

KEY WORDS — carotid artery, iodine 125, recurrent squamous cell carcinoma, ultrasound.

INTRODUCTION

A significant population of head and neck cancer patients fail their initial treatment and present with extensive local recurrence. The disease often encases the vascular bundle, infiltrates along surgical planes, or invades bone. Surgical treatment alone is not curative because of the high likelihood of microscopic residual disease, and additional external beam irradiation can lead to unacceptable complications.

There have been several recent series on the use of reoperation and intraoperative iodine 125 (^{125}I) seed implantation with improved survival and local control and acceptable complication rates. This paper retrospectively reviews our experience using aggressive salvage reoperation and intraoperative ^{125}I seed implantation in the treatment of advanced recurrent squamous cell carcinoma of the head and neck. Ultrasound was utilized to examine the long-term effects of ^{125}I on the carotid artery.

MATERIALS AND METHODS

Medical records from April 1988 to June 1992 from the Departments of Otolaryngology–Head and Neck Surgery and Radiation Oncology at Temple University Hospital were reviewed. Twenty-five patients who had undergone ^{125}I seed implantation and reoperation were identified. One patient had a malignant sweat gland tumor and was excluded. The remaining 24 patients had recurrent squamous cell carcinomas confirmed histologically. Of the 24 patients, there were 5 women and 19 men with a mean age of 62 years (range 31 to 76). Patients were selected for combined implantation and surgery on the basis of the five criteria listed in Table 1. A detailed summary of each patient's original tumor and recurrence site is listed in Table 2. All patients had recurrent disease and satisfied at least one criterion. Involvement of the carotid artery was evaluated by computed tomography (CT) scan, magnetic resonance imaging (MRI), and/or ultrasound. In addition to resection and implantation, 17 of 24 (71%) patients had pectoralis major muscle used for reconstruction of the defect, protection of the carotid artery, and coverage of ^{125}I seeds. The distribution and average area covered are summarized in Tables 3 and 4. Patients were followed up for a minimum of 17 months or until time of death. The median survival was 7 months (range 1 to 51 months). At the termina-

TABLE 1. SELECTION CRITERIA

Failed previous irradiation
Failed previous surgical resection
Failed previous chemotherapy
Tumor involving carotid
Tumor involving skull base, pharyngeal wall

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TABLE 2. DETAILED SUMMARY OF EACH PATIENT'S ORIGINAL TUMOR AND RECURRENCE SITE

Pt. No.	*Age*	*Sex*	*Original Tumor*	*Stage*	*Original Treatment (Date of Completion)*	*Recurrence Site (Date)*	*DFI*
1	57	M	T2N1MX retromolar trigone	III	Composite resection followed by 6,000 cGy (1/88)	L pharynx, skull base (5/88)	4
2	57	M	T3N2M0 base of tongue cancer	IV	Chemo and XRT with 7,029 cGy to primary tongue, and both upper neck with 8,000 cGy (10/90)	L neck, residual carotid nodes (8/91)	10
3	63	F	T2N0M0 tonsillar cancer	III	Preop XRT and surgery (11/89)	R neck involving carotid (11/90)	12
4	70	F	T2N0M0 tongue cancer	II	Initial local excision and then first neck recurrence treated with XRT: 6,440 cGy (6/90)	R carotid (2/91)	8
5	55	F	T1N3M0 tonsillar cancer	IV	Surgery and unknown XRT (1/91)	L carotid (11/91)	10
6	79	F	T1N0 posterior pharyngeal wall cancer	I	Unknown XRT (2/88)	L pharynx and carotid (12/88)	10
7	65	M	Laryngeal cancer and floor of mouth		Surgery and XRT (8/89)	Floor of mouth (10/89)	2
8	41	M	T1N1M0 R vallecula cancer	III	4,500 cGy as combined preop XRT and surgery but never completed surgery (9/87)	R neck involving carotid (9/88)	12
9	55	M	T3N3M0 tongue cancer	IV	6,000 cGy and surgery (9/88)	R carotid (3/89)	6
10	32	M	Recurrent tongue cancer	IV	Surgery and XRT: 7,600 cGy to tongue, and 5,000 cGy to upper neck (9/89)	L neck and tongue (1/90)	4
11	76	M	T2N1MX R base of tongue cancer	III	XRT: 6,000 cGy in 1988 and chemo (11/89)	R neck with carotid (7/90)	8
12	75	M	Laryngeal cancer		XRT: 6,000 cGy and chemo (2/90)	Retropharyngeal prevertebral (6/91)	16
13	66	M	L neck mass	IV	Treated primarily with ^{125}I	Carotid	N/A
14	53	M	T1 laryngeal cancer	IV	Chemo and XRT previously for lymphoma in neck and vocal cord lesion, then 9/88 had XRT and laryngectomy for stoma recurrence, then submental mass recurrence (2/89)	Hypopharyngeal and L carotid (9/89)	7
15	70	M	R tonsillar cancer		Treated with 7,000 cGy in 1980 (10/86)	R neck with carotid involvement (10/91)	60
16	60	M	T1N0 supraglottic cancer	I	Surgery and XRT (6/91)	Surgical bed carotid (3/92)	9
17	58	M	T3N2MX laryngeal cancer	IV	Chemo and XRT with surgical salvage (11/87)	L neck with carotid (10/90)	35
18	57	M	SCCA of parotid		XRT (2/90)	Skull base (4/90)	2
19	59	F	Base of tongue cancer	IV	Surgery and XRT (2/85)	Posterior pharynx, R carotid (5/89)	51
20	71	M	T3N0 R vocal cord cancer	III	6,400 cGy larynx, then total laryngectomy (7/88)	L neck with carotid (5/89)	10
21	75	M	T3N0 epiglottic cancer	III	XRT and surgery (5/85)	R neck with carotid (11/87)	30
22	68	M	T2N0M0 laryngeal cancer	II	7,200 cGy in 1989 (8/89)	R carotid (7/91)	23
23	63	M	T4N2M0 L base of tongue cancer	IV	Surgery and XRT (4/91)	R neck with carotid (1/92)	9
24	71	M	T2N0M0 laryngeal cancer	II	Surgery and XRT (8/89)	L mandible and neck with carotid (6/91)	22

DFI — disease-free interval in months from original treatment, chemo — chemotherapy, XRT — radiotherapy, SCCA — squamous cell carcinoma, N/A — not available.

TABLE 3. DOSES OF IODINE 125 IMPLANTS

Isodose Surface (cGy)	No. of Pts. (N = 23)*	Average Area (cm^2; Coronal/ Sagittal)	Range (cm^2; Coronal/ Sagittal)
5,000	23	52/52	20-122/12-94
10,000	23	28/29	9- 79/ 7-64
15,000	9	23/21	6- 41/ 7-57
20,000	5	16/13	2- 34/ 8-19
30,000	1	4/ 3	N/A

N/A — not applicable.

*One patient's radiation isotope curve was not available for analysis.

TABLE 4. EXTERNAL BEAM IRRADIATION

	Pre-^{125}I Irradiation	Post-^{125}I Irradiation
No. of patients	22*	10†
Average radiation dose (cGy)	6,320	4,250
Range (cGy)	4,500-7,600	3,000-6,950

*Three patients' data were not available for analysis.

†Two of these patients did not receive pre-^{125}I irradiation.

tion of follow-up, patients were identified as with or without evidence of disease. Survival statistics were analyzed by the method of Kaplan and Meier.[1]

All ^{125}I seed sources were ordered from Medi-Physics, Inc (model 6711, Arlington Heights, Ill) at least 3 days before implantation. All seeds were spaced at a fixed 1-cm distance within No. 1 Vicryl (polyglactin 910) absorbable suture.

After informed consent, all patients underwent "extended radical neck dissection" with or without primary tumor resection and coverage of the carotid artery with a levator scapula muscle flap or a pectoralis major muscle flap, usually without a skin paddle. The outer surface of the pectoralis muscle was usually covered with a split thickness skin graft to avoid torsion of the vascular pedicle. Tumor resection was accomplished (no gross visible disease), and as much as possible of the wound was closed and flaps were inserted. Polyglactin sutures containing the ^{125}I seeds threaded on a separate curved Keith needle (Richard Allen Abdominal cutting) were then sewn into the tumor bed with the assistance of the radiation oncologist (C.L.S.). The seeds were approximately 0.5 mCi per seed strength. The probable number of seeds to be used was determined preoperatively by joint discussion between the surgeon (R.M.M.) and radiation oncologist. A minimum dose of 10,000 cGy was to be delivered to the tumor bed. The surgical technique of seed implantation was tailored to the individual patient. The seeds were sewn in regularly spaced intervals, 0.7 to 1 cm between each row. The area covered by the implant was determined individually on the basis of operative findings of both the surgeon and oncologist. Each row of sutures was initially placed such that the end containing the ^{125}I seeds remained in the shielded lead loop until the end of the row was reached. After confirmation of hemostasis, the wound was then closed in standard fashion and the seed count was documented in the operating room by portable x-ray. Postoperative care was routine with the exception of radiation safety precautions. After being judged medically stable, postoperative patients were brought to the radiation oncology department for orthogonal films to document seed position and dosimetry. At the time of discharge, a lead shield and written instructions were provided for the patient and family. Selected patients received additional external beam radiotherapy as outpatients if it was deemed necessary and safe.

From the orthogonal films, isodose curves were calculated from the Brachytherapy program version 2.7 on a CMS computer. The area (in square centimeters) of each isodose level was then calculated in both the coronal and sagittal planes.

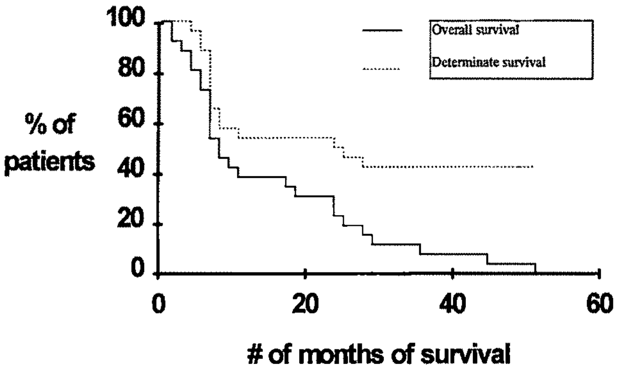
Carotid real-time ultrasonography was obtained on selected patients. The ultrasonography was carried out by the Department of Neurology neurovascular laboratory. Multiple transverse and longitudinal sections were obtained of the common, internal, and external carotid arteries.

RESULTS

Table 5 summarizes the distribution of patients fulfilling selection criteria. Radiotherapy failure was the most common criterion. In addition, most patients fulfilled second and third criteria. Carotid sheath involvement with tumor was found in 19 of 24 patients. The average implantation areas required in coronal and sagittal planes were 28 and 29 cm^2, respectively.

TABLE 5. SUMMARY AND DESCRIPTION OF CRITERIA FULFILLED

	No. of Pts.	No. of Criteria Fulfilled
XRT, surgery, chemo, carotid	2	4
XRT, surgery, carotid, skull base	1	4
XRT, surgery, skull base	1	3
XRT, chemo, carotid	5	3
XRT, surgery, carotid	6	3
XRT, carotid, pharynx	2	3
XRT, chemo, pharynx	1	3
XRT, surgery	1	2
XRT, carotid	3	2
Surgery, skull base	1	2
Carotid	1	1



Kaplan-Meier survival curve.

At the end of the analysis, 7 of 24 patients (29%) remain alive. Eleven of 24 patients (46%) were free of disease at 2 years or at time of death. The overall 2-year survival was 29% with the determinant survival rate of 50%. Fourteen of 24 patients were found to be with disease locally (primary or neck), and 2 are alive. Of the 10 patients without disease, only 5 are still alive. The complete survival results are shown in the Figure.

Complications following surgical resection occurred in 12 of 24 (50%) patients and 10 of 17 (59%) undergoing flap reconstruction. The most common wound-related postoperative complication was infection and wound ulcers (25%). The second most common was seed exposure related to wound ulcer or flap dehiscence. One patient had flap dehiscence after falling down during the immediate postoperative period. Although 2 patients had carotid rupture, this did not occur until several months after the treatment, and both had massive local regrowth at the time of rupture. In addition, 4 of 24 patients (17%) acquired *Clostridium difficile* or methicillin-resistant *Staphylococcus aureus* during the hospitalization. The complications are summarized in Table 6.

TABLE 6. COMPLICATIONS

	No.	*%*
Seed exposure	3	13
Flap dehiscence	1	4
Radionecrosis	2	8
Fistula, infection, and ulcer	6	25
Clostridium difficile or methicillin-resistant *Staphylococcus aureus*	4	17
Carotid rupture	2	8

N = 12 out of 24.

Of the 7 surviving patients, 5 were available for evaluation of the carotid artery by ultrasound. The average time between ultrasound and treatment was 30 months (range 13 to 42 months). No patient exhibited clinical signs of carotid artery occlusive disease. Four of the 5 studied patients had no significant disease in the implanted carotid as compared to the nonimplanted side. One patient showed a mild encroachment of the lumen as compared to the nonimplanted side. The results are summarized in Table 7.

DISCUSSION

Treatment of patients with recurrent head and neck carcinoma continues to be a challenging problem. Patients are often judged unresectable or marginally resectable by preoperative CT, MRI, and ultrasound. There are difficulties in obtaining clear surgical margins because of involvement of the carotid artery,

TABLE 7. SUMMARY OF CAROTID ULTRASOUNDS

Age	*Site of Implantation*	*Interval of Carotid Study After Implant (mo)*	*Carotid Ultrasound Result*	*Maximum Dose Delivered to Carotid (cGy)*
34	L neck, carotid, tongue	33	L common carotid artery, bifurcation and internal carotid artery appear clear; R common carotid artery, bifurcation, internal and external carotid arteries are clear	Previous XRT 5,000 Iodine 125 10,000 Postop XRT 2,700
44	R neck	40	R and L common carotid artery, bifurcation, internal and external carotid arteries are clear	Previous XRT 4,500 Iodine 125 20,000
58	L neck, carotid	13	Mildly stenotic plaque formation within R common carotid artery and bifurcation; on L, mildly stenotic plaque formation is seen within bifurcation	Previous XRT 8,010 Iodine 125 15,000
58	R carotid	42	Carotid real-time ultrasonography and Doppler spectral analysis indicate mild encroachment of R carotid lumen; marked high echo density soft tissue abnormalities are seen on R; no obvious intraluminal stenosis is seen on L	Previous XRT 6,000 Iodine 125 10,000 Postop XRT 2,700
78	R neck, carotid	23	Nonstenotic plaque formation within origins of internal carotid arteries bilaterally	Previous XRT 6,000 Iodine 125 20,000 Postop XRT 3,000

TABLE 8. SUMMARY OF COMPARABLE STUDIES

Authors	*Year*	*Patient Population (N)*	*Isotopes and Techniques*	*Complication Rate*	*Survival*
Vikram et al[5]	1983	Primary or palliation (124)*	^{125}I single seed applicator	5.5% serious	9% 2 y and 5.5% 5 y
Martinez et al[6]	1983	Primary and recurrent (48)	^{125}I single seed applicator or polyglactin suture carrier	11%	17% 2.5 y for recurrent group, 50% 5 y for primary group
Fee et al[7]	1983	Primary and recurrent (29)	^{125}I polyglactin suture carrier	41% overall, 28% major	15 mo for primary group, 12 mo for recurrent group
Paryani et al[8]	1985	Primary and recurrent (38)	^{125}I polyglactin suture carrier	26% overall	11 mo
Son and Ariyan[9]	1985	Primary or recurrent (6)	^{125}I single seed applicator to primary, and polyglactin suture carrier to neck	33%	4 mo to 1 y with no evidence of disease
Son and Kacinski[10]	1987	Primary (14)	^{125}I or ^{192}Ir single seed applicator	18% in ^{125}I group	82% actuarial 5 y in whole group
Park et al[11]	1991	Recurrent (35)	^{125}I single seed applicator	36% significant	38% 2 y
Present study		Recurrent (24)	^{125}I polyglactin suture carrier	21% major, 50% overall	29% 2 y

*Eighty-nine percent of cases were epidermoid.

skull base, or deep neck musculature and/or previous radiotherapy.[2,3] Iodine 125 is a desirable radionuclide for implantation because of its ease of handling and low complication rate. Because of tissue attenuation of the low-energy gamma rays produced by ^{125}I, a high therapeutic ratio of tumor and previously irradiated tissue can be achieved.[4] Several studies of improved survival and local disease control have been reported using surgical resection of gross tumor combined with ^{125}I to eradicate microscopic disease in advanced head and neck cancer.

Vikram et al[5] treated 124 patients with advanced head and neck cancer with palliative intent using permanent ^{125}I implants. Eighty-nine percent of the histologic types were epidermoid carcinoma, with the remaining distributed among salivary gland and thyroid carcinomas. With a single seed applicator, ^{125}I seeds were inserted into the tumor bed. Complete regression occurred in 71%, partial regression in 18%, and no regression in 11%. Overall, 64% of patients remained locally controlled until the patients' deaths. Only 9% of patients survived for 2 years, and 5.5% survived for 5 years. The rate of serious complications was 5.5%.

Martinez et al[6] attempted to increase local control rates using ^{125}I seeds either individually placed or inserted into absorbable polyglactin suture carriers. Forty-eight patients with either locally advanced cancer, massive cervical lymphadenopathy, or locally recurrent head and neck cancers were treated. The overall local control rate was 58%, and the 5-year survival of those treated for cure was 50%. For those with recurrent disease, only 17% survived for 2.5 years. The complication rate was 11%.

Fee et al[7] treated 29 patients with large neck carcinomas attached to the carotid artery. Implantation of ^{125}I via an absorbable suture and surgical resection were utilized with curative intent. Eighteen patients out of 29 were treated for recurrent neoplasm. Overall, 62% of patients were disease-free in the neck with a 1-year follow-up. The mean survival was 15 months in the primary treatment group, and 12 months in the recurrent group. The overall complication rate was 41%, and the major complication rate was 28%.

Paryani et al[8] treated 38 patients with advanced head and neck cancer attached to the carotid artery using surgical excision followed by ^{125}I polyglactin suture implant in the neck. Twenty-six patients out of 38 underwent implant for recurrent disease. The local and regional control rate was 53%. The mean survival was 11 months. The overall complication rate was 26%.

Son and Ariyan[9] reported preliminary results. They treated 6 patients with recurrent or metastatic head and neck tumors with surgical resection and a combination of single seed implantation to the primary and polyglactin ^{125}I sutures to the neck. All 6 patients were locally free of disease for 4 months to 1 year.

Son and Kacinski[10] treated 14 patients with primary pharyngeal wall cancers using external beam and interstitial implants (either ^{125}I or ^{192}Ir). Of the 14 patients, 9 were implanted with a single seed ^{125}I applicator. Of the 9 patients, all achieved local control. The complication rate for the group of 9 was not reported, but overall for the whole group it was 18% with reversible soft tissue and mucosal injury.

Park et al[11] treated 35 patients with recurrent squamous cell carcinomas of the head and neck using surgical resection and a ^{125}I single seed applicator. The local control rate was 29% at 5 years. The significant complication rate was 36% for all cases. Table 8[5-11] summarizes the above studies.

All of the above studies suggested promising results for ^{125}I implantation combined with surgical excision. Each of the studies is different in patient population (primary versus recurrent), technique, and criteria of complication. Our study was most similar to the study by Park et al[11] in that all patients had failed previous therapy, and the complication categories were similar. Our study differs from that of Park et al by the technique of ^{125}I insertion. Analysis of our data shows that the 2-year overall survival of 29% and determinant survival of 50% are similar to those in the study by Park et al: 38% and 47%, respectively. However, our overall complication rate appears slightly higher in comparison: 50% versus 36%. This may be related to our higher percentage of patients requiring flap reconstruction. The complication rate was 59%, compared to 56% in the study of Park et al when flap reconstruction was used. In addition, their technique of implantation also differs by polyglactin suture versus single seed applicator. Although our complication rate appears high, we feel most of the complications are related to the presence of the recurrent or persistent disease rather than the ^{125}I implants.

Within the complication group, 2 patients had carotid rupture. Both patients were known to have extensive local tumor recurrence. It was unclear whether the first rupture occurred within the implant volume. The second patient had methicillin-resistant *S aureus* tracheitis and a small area of >30,000 cGy within the tumor bed. For both, the specific cause for the rupture is unknown.

Carotid resection may avoid carotid rupture; however, it is not a viable option for all patients. Dissection of recurrent tumor in the neck often requires close implantation adjacent to the carotid artery or stump. Other series in the literature have reported the relative safety of such implants. In a study by Fee et al,[12] only 1 of 38 patients developed carotid rupture and had proven local recurrence at the site. In the study by Park et al,[11] 2 of 35 patients had carotid rupture and also had proven local recurrence at the site. Using animal models, Fee et al[12] have shown that there are no significant effects to the carotid artery in the short term; however, the long-term effect is still unclear. Local recurrence of tumor near the carotid appears to be a common denominator for predisposing to rupture. Other factors, such as excessive irradiation and infection, may also contribute. To evaluate possible long-term complications relating to the carotid artery involvement, we studied the effect of brachytherapy on the carotid using ultrasonography. Ultrasound offered a noninvasive yet accurate method to evaluate the patency of the irradiated carotid arteries. Each patient's carotid artery received at least 10,000 cGy from the ^{125}I implants in addition to any preoperative or postoperative irradiation. All studies occurred at least 1 year from time of implantation. Using the nonimplanted side as a control, we found that there were minimal or no changes on the implanted side. We feel that our carotid ultrasound results support the canine data by Fee et al,[12] and ours is the only such study in humans.

CONCLUSIONS

Despite significant advancement in the treatment of head and neck squamous cell carcinoma, a significant population of patients presents with recurrent disease. These patients often require aggressive multimodality treatments. With salvage surgery and intraoperative ^{125}I implantation, complication rates are acceptable and survival rates are improved. Patients who do best have microscopic disease, do not require pharyngeal entry, and have recurrence confined to the neck. Iodine 125 is relatively safe in the short term and long term when implanted around the carotid artery.

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Ann Otol Rhinol Laryngol 105:1996

STABLE AND PROGRESSIVE HEARING LOSS IN TYPE 2A USHER'S SYNDROME

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Audiograms were traced or additionally performed on 23 Usher's syndrome patients in 10 Dutch multi-affected families, all linked to chromosome 1q (USH2A locus). Serial audiograms, available in 13 patients, were used for a regression analysis of binaural pure tone average on age (follow-up, 9 to 32 years) to test for "significant progression," ie, a significant regression coefficient, here called the "annual threshold increase" (ATI, expressed in decibels per year). A significant ATI (>1 dB/y) was observed in 3 patients. Analysis of variance of ATI demonstrated significant heterogeneity; hearing loss was either stable or progressive. This implies a significant clinical heterogeneity. A similar analysis performed on our progressive USH2A cases and "type III" cases previously reported by others (ATI of 1 to 5 dB/y), some of which were recently linked to chromosome 3q (USH3 locus), failed to show any significant heterogeneity in the progression of hearing loss.

KEY WORDS — deaf-blindness, gene linkage, genetic deafness, progressive deafness, retinitis pigmentosa, sensorineural hearing loss, type III Usher's syndrome.

INTRODUCTION

Usher's syndrome is an autosomal recessive disorder characterized by congenital sensorineural hearing loss (SNHL) that is combined with progressive pigmentary retinopathy that shows onset in childhood or adolescence and leads to severe constriction of the visual field (tunnel vision). Cataracts usually occur later and cause additional loss of vision, eventually up to the point of (sub)total blindness.

Three clinical subtypes were currently distinguished, before the gene linkage studies started.[1-3] Type I involves profound congenital SNHL that causes abnormal speech and language development, combined with vestibular areflexia or severe hyporeflexia, which later in life adds to the problem of spatial disorientation as the visual system further deteriorates. Type II involves moderate to severe SNHL, most pronounced at the higher frequencies, whereas at the speech frequencies there generally is sufficient hearing ability to allow for the normal development of speech and language. Vestibular (caloric) responses can be elicited. The SNHL of this type was defined as stationary throughout life, apart from the development of presbycusis at a more advanced age. A clinical type of Usher's syndrome with features similar to type II, in which, however, the SNHL proved to be progressive, was designated as type III.

Gene linkage studies have split type I into three subtypes and type II into two subtypes. Type I has been linked to loci at 14q32 (USH1A),[4-6] 11q14 (USH1B),[5,6] and 11p13-15 (USH1C),[6] whereas type II has been linked to 1q41 (USH2A).[7-9] Usher's cases that fail to show linkage to chromosome 1q41 are classified as Usher's type 2B.[10,11]

This study shows the long-term results of audiometry in 23 patients from 10 Dutch multi-affected families with Usher's type 2A syndrome. Serial audiograms were collected and analyzed to evaluate whether the hearing loss was stable or showed significant progression.

PATIENTS AND METHODS

Our study population comprised 23 patients from 11 different sibships in 10 kindreds with more than 1 affected person. Blood samples from affected persons and their nonaffected relatives whose medical

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From the Departments of Otorhinolaryngology (van Aarem, Huygen, Cremers) and Ophthalmology (Pinckers), University Hospital Nijmegen, Nijmegen, and the Netherlands Ophthalmic Research Institute, Amsterdam (Bleeker-Wagemakers), the Netherlands, and Boys Town National Research Hospital, Omaha, Nebraska (Kimberling). This study was supported in part by grants from Stichting De Drie Lichten, Stichting Het Heinsius-Houbold Fonds, Landelijke Stichting voor Blinden en Slechtzienden, Nederlandse Stichting voor het Gehandicapte Kind, and the Algemene Nederlandse Vereniging ter Voorkoming van Blindheid, the Netherlands. The gene marker studies were supported by National Institutes of Health grant NIDCD PO1-DCO1813-01 and grant 89-198 from the Retinitis Pigmentosa/Fighting Blindness Foundation.

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TABLE 1. SUMMARY DIAGNOSTIC AND LINKAGE DATA FOR EACH MULTIPLEX SIBSHIP OR KINDRED ANALYZED

Family	*Probability of Linkage to 1q41 Markers*	*Tentative Audiologic Phenotype*
A	.985	II
B	.980	II
C	.972	III
D	.979	II
E	.979	II
F	.997	Mixed
G	.972	II
H and I	.993	Mixed
K	.996	Mixed
M	.955	II

Phenotype II is stable hearing loss. Phenotype III is progressive hearing loss. Mixed phenotype, phenotype III, does occur, but not — with at least some certainty — in all sibs.

history indicated stable hearing loss (type II) were collected and analyzed at Boys Town National Research Hospital for gene linkage. The following chromosome 1 markers had been typed on all family members: D1S245, D1S70, D1S217, D1S237, D1S229, D1S227, PPOL, and D1S81. The Usher's 2A gene is flanked by a 2.4 centimorgan region bounded by D1S237 and D1S229.

The patient's history was taken and a general otorhinolaryngological examination, including audiometry and collecting previous audiograms, was performed with otoscopy in all instances, and the diagnosis of Usher's syndrome was confirmed by one ophthalmologist with assessment of the extent of retinitis pigmentosa, impairment of the visual fields, visual acuity, dark adaptation testing, and an electroretinogram. Vestibular excitability was confirmed with rotatory tests and caloric tests. This report will be limited to the evaluation of the audiometric data.

Audiometric data could be obtained from the files from the Nijmegen otorhinolaryngology department or elsewhere. In all cases clinical routine or serial audiometry was performed according to common standards and with the usual clinical equipment. Serial audiometry over 9 years up to 32 years was available in 13 patients. Most of the patients were also examined in the University Hospital Nijmegen, where pure tone audiograms were measured with an Interacoustics AC5 audiometer (Interacoustics, Assens, Denmark), calibrated following the ISO 389[12] procedure according to the ISO 8253-1 standard[13] over the most recent years. The following tentative audiologic phenotypes were distinguished: type II (stable hearing loss), type III (progressive hearing loss), and "mixed" (different types occurring simultaneously in one family). The assignment of each sibship was established by inspecting plots of the binaural pure tone average (BPTA, in decibels hearing level [HL]) against age; BPTA is the average of the pure tone averages (PTAs, at the frequencies 0.5, 1, and 2 kHz) of both sides. This tentative phenotype was used to test in first approximation whether it discriminated between the linked and unlinked subtypes of Usher's type II. Statistical tests on audiologic data comprised a regression analysis of BPTA on age to see whether the regression coefficient — here called "annual threshold increase" (ATI) and expressed in decibels per year — differed significantly from zero in each individual with sufficient ($n \geq 3$) serial audiologic data available, and an analysis of variance of the ATI values to test for (non)homogeneity in type of hearing loss.

TABLE 2. MULTIPOINT LOD SCORES OF DUTCH FAMILIES GROUPED BY AUDIOLOGIC PHENOTYPE

Group	*Family*	*Distance From D1S237 (centimorgans)* –10	0	+2	+12
II	A, B, D, E, G, M	2.485	3.535	3.463	2.438
III	C	0.414	0.582	0.683	0.415
Mixed	F, H and I, K	2.800	3.834	3.876	2.838
Total		5.699	7.951	8.022	5.691

RESULTS

There were 11 different families and 10 different kindreds (the families H and I were the same kindred) with Usher's type 2A. Two of the families involved multiple sibships of affected persons and the lod score data for these families represent linkage analysis on the whole kindred and are not presented on an individual sibship basis. The heterogeneity analysis (Ott's A-test) was employed to determine the probability that each family was linked to a chromosome 1q marker set. The overall χ^2 was nonsignificant when only the 10 Dutch families were included, and the resulting individual probabilities of each family being type 2A were all estimated at 1.0. Only when the Dutch data were pooled with the total set of 68 Usher II families ascertained worldwide were conservative probabilities obtained (the overall rate of heterogeneity was estimated at 12.5%). The resulting probabilities are shown in Table 1 and indicate that none of the families showed convincing nonlinkage with 1q41 markers. Family M had the lowest probability, .955, because it was the least informative of all the families.

As a further test of heterogeneity, the families were divided into three groups, those in which most members fit with the II phenotype with a stable hearing loss (families A, B, D, E, G, and M), those fitting the

TABLE 3. HEARING LOSS, MEASURED BY BINAURAL PURE TONE AVERAGE, AND REGRESSION ON AGE AND ASSIGNED TENTATIVE AUDIOLOGIC PHENOTYPE

				Regression Parameters		
Subject	*Sex*	*Age and Binaural Pure Tone Average*	*Audiologic Phenotype*	*r*	*ATI (dB/y)*	*SD (dB/y)*
A1	F	43 y 0 mo 60 dB HL; 48 y 11 mo 64 dB HL	II			
A2	M	31 y ? mo 54 dB HL; 44 y 11 mo 65 dB HL	II			
B3	M	55 y 9 mo 34 dB HL	II			
B4	M	24 y 5 mo 32 dB HL; 40 y 6 mo 42 dB HL; 40 y 10 mo 48 dB HL	II	.94	0.805	0.305
C5	F	21 y 3 mo 69 dB HL; 26 y 5 mo 73 dB HL; 31 y 10 mo 82 dB HL; 36 y 10 mo 84 dB HL	III	*.97*	*1.04*	0.172
C6	M	11 y 3 mo 52 dB HL; 13 y 5 mo 61 dB HL; 22 y 1 mo 78 dB HL; 28 y 1 mo 82 dB HL; 33 y 7 mo 87 dB HL; 38 y 0 mo 88 dB HL	III	*.95*	*1.31*	0.204
D7	M	11 y 4 mo 48 dB HL; 26 y 5 mo 48 dB HL; 27 y 3 mo 53 dB HL	II	.54	0.174	0.271
D8	F	13 y 4 mo 47 dB HL; 22 y 0 mo 48 dB HL; 24 y 2 mo 48 dB HL	II	.98	0.099	0.019
E9	F	6 y 10 mo 49 dB HL; 24 y 3 mo 56 dB HL; 25 y 3 mo 58 dB HL	II	.99	0.450	0.075
E10	M	5 y 7 mo 35 dB HL; 13 y 3 mo 33 dB HL; 20 y 10 mo 38 dB HL	II	.59	0.196	0.266
F11	F	5 y 7 mo 48 dB HL; 10 y 7 mo 48 dB HL; 14 y 4 mo 54 dB HL; 26 y 8 mo 59 dB HL; 37 y 3 mo 87 dB HL	III	*.93*	*1.17*	0.272
F12	F	32 y 5 mo 67 dB HL; 34 y 9 mo 62 dB HL	II or III			
F13	F	15 y 0 mo 57 dB HL; 20 y 1 mo 64 dB HL; 25 y 11 mo 64 dB HL	II or III	.85	0.626	0.395
G14	F	39 y 9 mo 61 dB HL; 44 y 6 mo 62 dB HL; 48 y 9 mo 62 dB HL; 48 y 11 mo 61 dB HL	II	.31	0.041	0.090
G15	M	45 y 8 mo 58 dB HL; 50 y 9 mo 66 dB HL	II			
H16	F	45 y 4 mo 88 dB HL; 50 y 3 mo 89 dB HL	III?			
H17	F	42 y 10 mo 81 dB HL; 45 y 10 mo 86 dB HL	III?			
I18	M	15 y 7 mo 48 dB HL; 22 y 8 mo 54 dB HL	II or III?			
I19	F	5 y 4 mo 50 dB HL; 11 y 5 mo 48 dB HL; 12 y 0 mo 46 dB HL; 21 y 3 mo 53 dB HL	II	.52	0.235	0.275
I20	M	6 y 11 mo 50 dB HL; 7 y 3 mo 48 dB HL; 20 y 3 mo 56 dB HL	II	.97	0.529	0.143
K24	F	40 y 2 mo 58 dB HL; 47 y 8 mo 68 dB HL; 49 y 10 mo 61 dB HL; 51 y 9 mo 65 dB HL	II	.80	0.695	0.530
K25	F	41 y 10 mo 95 dB HL	III?			
M29	M	33 y 6 mo 58 dB HL; 38 y 6 mo 62 dB HL	II			

Annual threshold increase (ATI) equals regression coefficient (correlation coefficient r); SD is its standard deviation. Italics indicate significant difference from zero.

III phenotype with a progressive hearing loss (family C), and those that seemed to be mixtures of the two phenotypes (families F, H and I, and K). The lod scores for these three groups were summed at constant positions −10, 0, +2, +12 centimorgans relative to marker D1S237, and the results are listed in Table 2. An M-test analysis (Morton) was carried out on the grouped data. The result was $\chi^2(2) = 0.20$ (not significant).[14] Thus, it is concluded that dividing the sample into different audiologic phenotypes does not uncover any genetic heterogeneity of Usher's type II. Thus, we would conclude that this clinical variability is not due to the involvement of two (USH) loci.

The available audiometric measurements are presented by age in Table 3; included is the assigned tentative audiologic phenotype. The mean difference between right and left PTA was only about 5 dB (SD about 4 dB). A systematic study of the separate sound frequencies showed that the progression of hearing loss (when present) could be regarded as being sufficiently represented by the progression at the speech frequencies (0.5, 1, and 2 kHz), especially at 1 kHz. It was thus validated that the PTA (and hence the BPTA) was a suitable measure of the development of hearing loss.

Those patients (n = 13) were selected from whom sufficient serial audiograms were obtained to analyze the regression of BPTA on age. The ATI (expressed in decibels per year) is the regression coefficient. An example of serial audiograms is shown in Fig 1. Histograms and probit plots (not shown) indicated the existence of two separate clusters of ATI values, those pertaining to cases without apparent progression (tentative type II, with a low ATI) and those pertaining to cases with a substantial ATI (tentative type III).

There were 3 patients with an ATI value that differed significantly from zero; ie, they showed

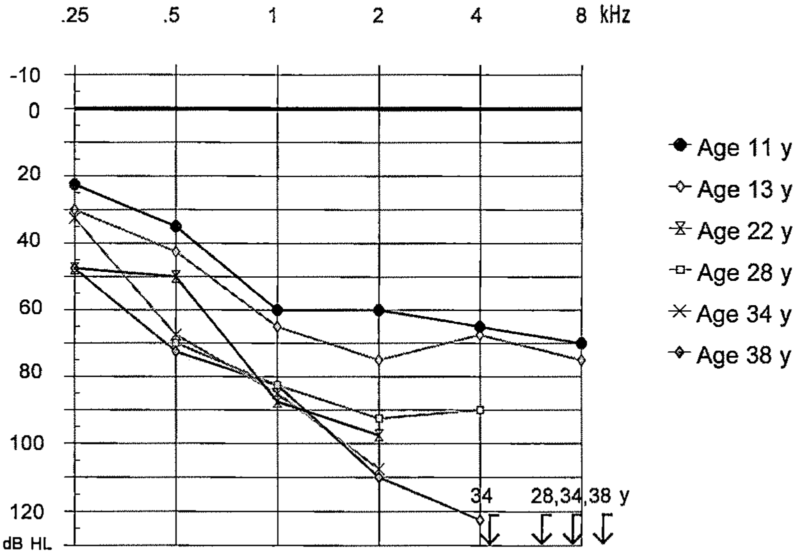

Fig 1. Serial audiograms of subject C6. Mean of right and left hearing levels (air conduction threshold, decibels hearing level) is shown. Regression line (binaural pure tone average [BPTA] on age) calculated from these values is $y = 1.31x + 42.7$ ($r = .95$ for $n = 6$, $p = .0030$), which indicates annual threshold increase (ATI) of about 1.3 dB/y (Table 3).

significant correlation and regression (Table 3): C5, C6 (also see Fig 1), and F11; their ATI was in excess of 1 dB/y. The continuous fat lines connecting the separate data points for these 3 patients are shown in Fig 2 to illustrate the progressive character of their hearing loss.

Analysis of variance on all of our cases with an ATI value available ($n = 13$) demonstrated that these values showed significant heterogeneity ($F = 2.44$, *df* 12 and 22, $p = .033$). A similar analysis showed significant heterogeneity ($F = 2.01$, *df* 19 and 31, $p = .041$) also for the combination of the present group of patients with known ATI and the cases previously described by others for whom regression analysis could be performed and an ATI value could be obtained (Table 4[15-18]). All of the latter cases had a progressive type of hearing loss reported to be type III Usher's syndrome, except for case 1 of Karjalainen et al.[16] Of interest, an analysis of variance performed on the previously reported cases and our own cases (ie, C5, C6, and F11) for whom ATI was in the range of 1 to 5 dB/y, ie, those who showed the most convincing progression of hearing loss, failed to show any significant heterogeneity in ATI values ($F = 1.20$, *df* 6 and 15, $p = .36$; after the exclusion of the case of Gorlin et al[15] — because of its large SD — $F = 2.07$, *df* 5 and 14, $p = .13$).

DISCUSSION

There is little doubt that there is substantial variation in the Usher's 2A audiologic phenotype, and at least two different patterns are apparent: one stable to slowly progressive, ie, in keeping with normal presbycusis,[19] and another more rapidly progressive. Progression was not apparent from the medical history. According to the χ^2 test applied to the data presented in Table 2, the apparent audiologic phenotype did not appear to be associated with linked or unlinked subtypes. No evidence of families showing other than 1q linkage was, in fact, observed. However, the phenotyping used, and thus the grouping of the families, was somewhat arbitrary. We therefore also performed analyses of variance on the ATI variable, ie, for those patients for whom sufficient follow-up data were available. The latter analyses on

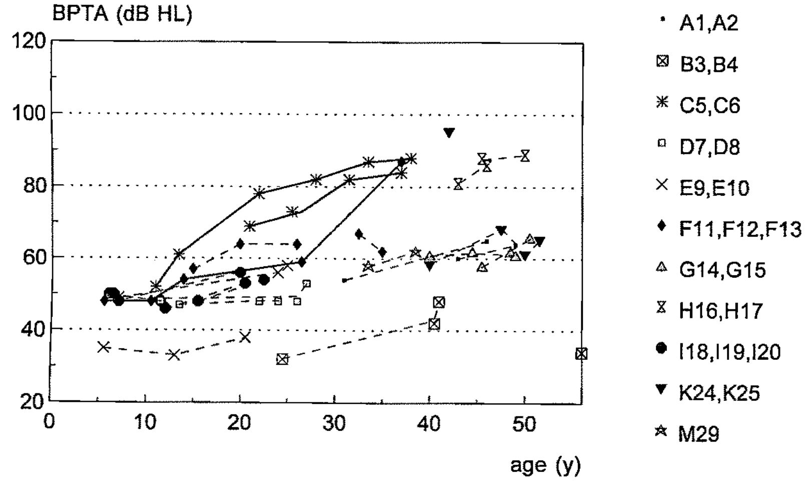

Fig 2. Plot of BPTA as measure of hearing loss against age for all of available data (Table 3). All separate data points are shown. Continuous fat lines connect individual longitudinal data in 3 patients with significant progression as apparent from their ATI (C5, C6, and F11). Dashed thin lines connect individual longitudinal data in other cases.

TABLE 4. BINAURAL PURE TONE AVERAGE AND REGRESSION ON AGE FOR PREVIOUSLY REPORTED PATIENTS FOR WHOM SUFFICIENT FOLLOW-UP DATA WERE AVAILABLE

					Regression Parameters		
Reference	*Case No.*	*Age and Binaural Pure Tone Average*	*Audiologic Phenotype*	*Genotype*	*r*	*ATI (dB/y)*	*SD (dB/y)*
Gorlin et al[15]	(One case)	4 y 57 dB HL; 5 y 85 dB HL; 9 y 90 dB HL	III		.75	5.07	4.41
Karjalainen et al[16]	Case 1	36 y 64 dB HL; 44 y 63 dB HL; 51 y 72 dB HL	II	USH3*	.79	0.518	0.405
	Case 3	9 y 52 dB HL; 18 y 59 dB HL; 32 y 72 dB HL	III	USH3*	*1.00*	*0.875*	0.041
	Case 4	10 y 86 dB HL; 24 y 102 dB HL; 31 y 105 dB HL	III	USH3*	.98	0.939	0.177
	Case 5	9 y 28 dB HL; 20 y 62 dB HL; 27 y 71 dB HL	III	?*	.98	2.45	0.487
	Case 7	5 y 63 dB HL; 13 y 77 dB HL; 17 y 113 dB HL	III	?*	.91	3.82	1.794
	Case 8	7 y 59 dB HL; 8 y 75 dB HL; 15 y 92 dB HL	III	?*	.92	3.50	1.443
Sankila et al[17]	Case II-1, family 15	7 y 55 dB HL; 8 y 80 dB HL; 10 y 97 dB HL; 18 y 98 dB HL; 18 y 108 dB HL	III	USH3	.80	3.08	1.338
Pakarinen et al[18]	Case II-5, family 7	35 y 73 dB HL; 42 y >100† dB HL; 51 y 115 dB HL	III	USH2A*		≈2.6	

Italics indicate significant values.

*Dr L. Pakarinen, personal communication 1995.

†Not measured at 2 and 4 kHz (pure tone average right ear).

the one hand clearly demonstrated heterogeneity in the progression of hearing loss in our patients, whereas on the other hand there was no significant difference in progression between our 3 cases with the most convincing progression and the similar cases previously reported for which sufficient data were available. Perhaps there is still lack of sufficient data, but it should be emphasized that 3 of our cases and 1 case previously reported (case 3 of Karjalainen et al[16]) showed significant progression.

The Dutch families are homogeneous with regard to linkage, and all are type 2A. Thus, the source of variation in audiologic phenotype seen here is not due to different mutations at different USH loci (insofar as presently known and therefore screened in our patients).

These results argue only that phenotypic variation in terms of the progression of hearing loss occurs within the Usher's 2A sample of families. There is only a single previous report that noted no difference between the hearing loss seen in type 2A versus 2B.[10] Thus, an investigation into possible audiologic differences between the type 2A and 2B subtypes is in order.

There are no linkage data published from families with the tentative clinical "type III Usher's," ie,

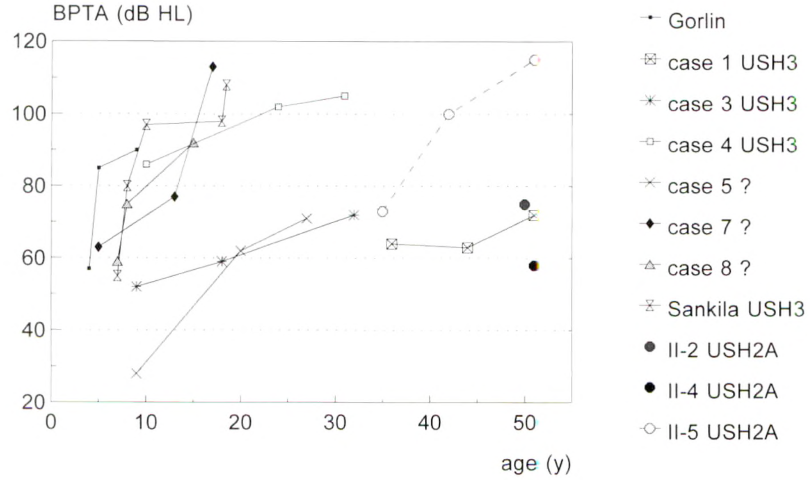

Fig 3. Plot of BPTA against age for previously reported patients, most of whom have disease designated as Usher's type III, with sufficient follow-up data to allow regression (solid lines) or trend (dashed lines) analysis (Table 4). Data for patients II-2, II-4, and II-5 were obtained from Dr L. Pakarinen (family 7, personal communication 1995).

progressive hearing loss, such as the patients described by Gorlin et al[15] and Karjalainen et al[16] whose hearing data were included in the present analysis (Fig 3 and Table 4), with one exception. Most recently, in the Finnish population Usher's syndrome type III has been linked to chromosome 3q (USH3 locus),[17] and some of the cases included in our analysis (Table 4 and Fig 3) recently turned out to be USH3. It seems that we have to face the situation that not only Usher's III patients but also Usher's 2A patients show, or may show, progression of hearing loss. Nevertheless, more detailed and more numerous data on the audiologic features of Usher's III patients, also based on gene linkage results, will be needed to conclude more precisely whether those types can be divided clinically only on audiometric data.

ACKNOWLEDGMENTS — We thank the patients and their relatives who participated in this study. We are also indebted to the Dutch Foundation for the Deaf and Blind and the Dutch Association of Usher's Patients for their help in contacting the families involved. Dr L. Pakarinen kindly provided the audiologic and linkage data on the Finnish patients.

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Ann Otol Rhinol Laryngol 105:1996

ROLE OF LEUKOTRIENE B4 AND INTERLEUKIN-8 IN ACUTE BACTERIAL AND VIRAL OTITIS MEDIA

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In order to evaluate the role of polymorphonuclear leukocytes (PMNs) in acute otitis media (AOM), levels of leukotriene B4 (LTB4), a potent inflammatory product of PMNs, and interleukin-8 (IL-8), a PMN chemotactic cytokine, were measured in 271 middle ear fluid (MEF) samples from 106 children with AOM. Forty-two percent of the patients had evidence of respiratory viral infection. At the time of diagnosis, levels of both LTB4 and IL-8 were higher in the MEFs from patients with AOM associated with bacterial or bacterial and viral infection than those MEFs containing no pathogen ($p < .05$). Antibiotic treatment was not associated with a significant change in levels of LTB4 or IL-8 in the MEFs obtained 2 to 5 days into treatment, compared to those obtained at diagnosis. Bacteriologic failure after 2 to 5 days of treatment was associated with high LTB4 levels in the initial MEFs ($p = .05$). Recurrence of AOM within 1 month was associated with high IL-8 levels in the initial MEF ($p = .04$). Our findings suggest that LTB4 and IL-8 are produced during acute infection of the middle ear, and these PMN-related inflammatory substances may play an important role in delaying recovery or in recurrence of AOM. Effective treatment of AOM may require eradication of bacteria by antibiotics, as well as pharmacologic agents that modulate PMN functions.

KEY WORDS — cytokine, interleukin-8, leukotriene, otitis media, respiratory disorder, viral disease.

INTRODUCTION

Acute otitis media (AOM) is one of the most common diseases in children. In spite of numerous effective antibiotics available for treatment of AOM, treatment failures, recurrences, and persistence of middle ear effusions still occur with high frequency.[1-3] A better understanding of the pathogenesis of the disease may help to develop strategies for effective management and prevention of the disease or its recurrences.

Viruses have been shown in the past decade to be important etiologic agents of AOM, either alone or in combination with bacteria.[4,5] Viruses can also interfere with bacteriologic and clinical responses to antibiotics in AOM.[6-8] One possible mechanism by which viruses interfere with response to treatment is that viral infection of the respiratory tract or the middle ear induces or enhances local synthesis of inflammatory mediators.[6] These mediators can impair phagocytic cell activity and cause increases in vascular permeability, mucous secretion, and fluid collection.[9]

In the early stages of acute inflammation of the middle ear subsequent to bacterial and viral invasion, polymorphonuclear leukocytes (PMNs) have been shown to play a major role.[10,11] We have previously shown in a guinea pig model that inhibition of PMN migration to the middle ear inoculated with bacteria is associated with reduced tissue injury.[12] However, the role of viral-bacterial interaction in PMN chemotaxis and activation needs further definition. In the present study, we measured levels of leukotriene B4 (LTB4), a potent inflammatory product released by PMNs, and interleukin-8 (IL-8), a potent PMN chemotactic cytokine, in middle ear fluids (MEFs) of children with AOM. The presence of these agents was then correlated with viral and/or bacterial causes, and with clinical and bacteriologic responses to antibiotics. Previous studies have shown that leukotriene (LT) concentrations in human MEFs are closely associated with the degree of inflammation and presence of bacteria in the middle ear.[13,14] Interleukin-8 has not previously been analyzed in MEF.

MATERIALS AND METHODS

Between December 1991 and January 1993, 122 children with AOM were enrolled in an antibiotic study. All children were generally healthy outpatients seen at our primary care clinics. Informed consent was obtained from parents or guardians. All procedures conformed to the guidelines established

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by the US Department of Health and Human Services and the University of Texas Medical Branch Institutional Review Board. The diagnosis of AOM was based on symptoms of fever, irritability or earache, signs of inflammation (red or yellow color or bulging) of the tympanic membrane, and the presence of MEF as documented by tympanocentesis. No patient had received an antibiotic during the previous week.

At enrollment (visit 1), tympanocentesis was performed and MEF was obtained for bacterial and viral studies. A nasal wash (NW) specimen and venous blood were also obtained for viral studies and serologic studies (acute titers), respectively. An NW specimen was obtained by flushing the nostril with 3 to 5 mL of phosphate-buffered saline solution in a 30-mL tapered rubber bulb. The procedure was performed after tympanocentesis. The patient was then randomly assigned to one of two antibiotic regimens: penicillin G benzathine and penicillin G procaine (Bicillin C-R) 0.6 to 1.2 million units intramuscularly, followed by 1 mL/kg per day of oral trimethoprim-sulfamethoxazole for 10 days, or ceftriaxone 50 mg/kg intramuscularly.

Follow-up visits were scheduled 2 to 5 days after initiation of therapy (visit 2), at the end of therapy (visit 3, days 9 to 12), and approximately 1 month after initiation of therapy (visit 4). At each follow-up visit, otologic examination was performed by one of the authors (V.M.H. or M.J.O.). During visit 2, a second tympanocentesis (MEF2) and a second set of viral studies were performed routinely. The patients who received ceftriaxone treatment received the second dose during this visit. Venous blood was obtained at visit 3 for determination of antibody responses to viruses (convalescent titers). The patient's condition was considered clinically improved when symptoms of AOM (fever, irritability, or earache) were alleviated, redness and bulging of the tympanic membrane were reduced or absent, and there was no drainage of MEF. Clinical failure was defined by continued inflammation of the tympanic membrane or purulent drainage, with or without bacterial growth in culture. The antibiotic was generally changed when clinical failure was observed. Bacteriologic failure was defined as isolation of pathogenic bacterium from MEF2, with or without clinical symptoms. Persistent otitis was diagnosed when clinical failure, bacteriologic failure, or both were observed at either visit 2 or visit 3. Response was considered good when clinical and bacteriologic successes were documented at visits 2 and 3. At visit 4 the presence of AOM was called a recurrence, although a few cases may have been persistent.

Specimen Processing and Virologic Procedures. Middle ear fluid was obtained by needle tympanocentesis as previously described.[15] Each sample was collected in a preweighed vial, which was reweighed to within 0.0001 mg. The specimen was processed immediately for aerobic bacterial cultures and susceptibility testing, by standard methods. The remaining MEF was diluted with 1.2 mL of phosphate-buffered saline solution. After inoculation into cell culture tubes, the diluted fluid was centrifuged to remove cellular elements, and the supernatant was frozen at −70°C for LTB4 and IL-8 assays.

Viral culture methods for MEF and NW specimens have been described previously.[8] In brief, the specimen was inoculated in two tubes each of primary monkey kidney cells, human fibroblasts (MRC-5), and HEp-2 cells. For each cell line, one tube was incubated at 33°C and the other at 36°C, and they were observed daily for 10 days for cytopathic effect. Hemadsorption was performed three times, and the final identification of viruses was done by standard methods.

Rapid viral antigen detection was performed on both MEF and NW specimens. For MEF, detection of respiratory syncytial virus (RSV) was performed by enzyme-linked immunosorbent assay (ELISA, Ortho RSV Antigen ELISA Test, Ortho Diagnostic Systems, Inc, Raritan, NJ). For NW specimens, cell pellets from centrifuged specimens were smeared onto microscope slides and used for rapid antigen detection by indirect fluorescent antibody techniques using commercially prepared antisera for RSV, influenza A and B viruses, parainfluenza viruses (types 1, 2, and 3), and adenoviruses (Bartels Viral Respiratory Panel, Baxter Healthcare Corp, West Sacramento, Calif). Viral serologic tests for the same respiratory tract viruses were performed by the indirect fluorescent antibody technique. Acute and convalescent sera from the same patient were tested at the same time, and a fourfold or higher rise in titers was considered a positive result.

LTB4 and IL-8 Assays. Quantitative determinations of LTB4 and IL-8 were performed on diluted samples of MEFs with commercial ELISA kits for LTB4 (Advanced Magnetics, Inc, Cambridge, Mass) and IL-8 (R & D Systems, Minneapolis, Minn). These assays utilize the "sandwich" immunometric technique. The absolute concentrations of LTB4 and IL-8 were calculated by comparison with internal LTB4 and IL-8 standards. For the LTB4 ELISA kit, the detection limit was 8.9 pg/mL, and cross-reactivity with LTC4, LTD4, LTE4, 5-hydroxyeicosatetraenoic acid (5-HETE), 12-HETE, and 15-HETE and with all prostaglandins was less than 1.0%. For IL-8, the detection limit was 4.7 pg/mL, and there was no

TABLE 1. NUMBER OF MIDDLE EAR FLUIDS AT FIRST VISIT CONTAINING BACTERIA, VIRUSES, OR VIRAL ANTIGENS

	Viruses					
Bacteria	*CMV*	*Influenza*	*Parainfluenza*	*RSV*	*No Virus*	*Total*
*Streptococcus pneumoniae**		1		1	29	31
*Hemophilus influenzae**				2	31	33
*Moraxella catarrhalis**					12	12
*Staphylococcus aureus**				3	2	5
Other bacteria†					4	4
Two bacteria			1		21	22
Three bacteria					6	6
Four bacteria					1	1
No bacteria	1	1	2	4	49	57
Total	1	2	3	10	155§	171

N = 171 from 106 patients; 45 (42%) patients had evidence of viral infection by antigen detection, culture, or serology.

CMV — cytomegalovirus, RSV — respiratory syncytial virus.

*Pure culture.

†2 *Hemophilus aphrophilus*, 1 *Streptococcus pyogenes*, and 1 *Pseudomonas aeruginosa*.

§Sixty-four of these middle ear fluid specimens were from patients with evidence of viral infection, documented by serology or detection of virus or virus antigen in nasal wash specimens. Viruses were those above, plus adenovirus, enterovirus, and rhinovirus.

cross-reactivity with other cytokines tested by the manufacturer. The LTB4 and IL-8 concentrations were standardized to weight (milligrams) of the MEF.

Statistical Methods. The LTB4 and IL-8 concentrations in the original MEF specimen (MEF1) were analyzed with SAS / PROC MIXED (SAS Technical Report P-229, SAS/STAT Software: Changes and Enhancements, 6.07, SAS Institute Inc, Cary, NC, 1992). The mixed procedure fits mixed linear models, including split-plot designs and repeated measures. The patient was specified as a random effect, the ear as a subunit with compound symmetry for the covariance structure, and the microorganism group as a fixed effect. Because of the unbalancedness in data structure, least-squares means were used for multiple comparisons. Although descriptive statistics in the Tables were computed from measurements, all analyses, including correlation, were conducted with logarithm-transformed data because of skewedness. Clinical and bacteriologic outcome variables were also analyzed with SAS/PROC MIXED. All tests of significance were assessed at the .05 level.

RESULTS

Study Population. One hundred twenty-two patients were enrolled in the antibiotic efficacy study. Of these, 106 subjects from whom MEFs were available for the LTB4 or IL-8 assay were included in this report. Forty-seven (44%) subjects were black, 34 (32%) white, 22 (21%) Hispanic, and 3 (3%) Asian. The ages ranged from 2 months to 4$^1/_2$ years (mean 17 months and median 13 months). There were 61 boys and 45 girls. Sixty-five patients had bilateral disease, and 41 had only unilateral disease. Of these, 3 patients did not return for a follow-up visit. The interval between enrollment (visit 1) and the first follow-up visit (visit 2) was 2 days in 58 patients, 3 days in 38 patients, and 4 or 5 days in 7 patients. One hundred seventy-one MEF1 samples were collected from 106 subjects, and 110 MEF2 samples were collected from 72 subjects.

Microbiologic Data. Virologic studies of the MEF, NW, and viral serologic studies separated the 106 patients into two major groups: those without evidence of viral infection (n = 61 or 58%), and those with acute viral infection (n = 45 or 42%). Forty-three patients were infected with a single virus: RSV,^{15} adenovirus,^{8} influenza A or B virus,^{7} parainfluenza viruses,^{5} cytomegalovirus (CMV),^{3} enterovirus,^{2} untyped picornavirus,^{2} and rhinovirus.^{1} Two patients were infected with both RSV and CMV. Virologic and bacteriologic data of 171 MEF1 samples are shown in Table 1. One hundred fourteen (67%) samples contained pathogenic bacteria with (8) or without (106) virus. Sixteen (9%) samples contained virus with (8) or without (8) bacteria. Forty-nine samples contained neither bacteria nor virus; however, 12 of these subjects had viral infection documented by NW or serologic studies.

LTB4 and IL-8 Data. One hundred seventy-one MEF1 samples from 106 subjects and 110 MEF2 samples from 72 subjects were available for LTB4 analysis. The LTB4 concentrations (picograms per milligram) in MEF1 ranged from 52 to 67,148 (mean 2,827, median 939). The LTB4 concentrations in MEF2 ranged from 41 to 123,318 (mean 5,433, median 1,383). One hundred thirty-six MEF1 from 91 subjects and 75 MEF2 from 55 subjects were

TABLE 2. LEUKOTRIENE B4 CONTENTS IN FIRST MIDDLE EAR FLUID SPECIMEN, RELATIONSHIP WITH MICROORGANISM IN SPECIMEN, AND CONCURRENT VIRAL INFECTION

			Leukotriene B4† *(pg/mg)*			*Interleukin-8*§ *(pg/mg)*		
Group	*Microorganism in MEF*	*Viral Infection**	*N*	*Median*	*Mean ± SE*	*N*	*Median*	*Mean ± SE*
1	Negative	–	37	431	1,080 ± 430	34	6,137	14,448 ± 3,868
2	Negative	+	12	1,069	3,340 ± 1,514	5	2,944	7,324 ± 3,504
3	Virus	±	8	581	828 ± 325	5	5,166	9,843 ± 6,216
4	Bacteria	–	54	969	4,712 ± 1,418	49	10,240	18,244 ± 3,259
5	Bacteria	+	52	1,107	2,323 ± 497	35	11,348	22,416 ± 4,275
6	Bacteria and virus	±	8	2,552	2,683 ± 580	8	52,646	49,057 ± 7,790

MEF — middle ear fluid.

*Detection of virus or viral antigen in nasal wash specimen or contralateral MEF, or positive viral serologic studies: – — no, + — yes, ± — yes or no, with positive virus in MEF.

†Statistically significant differences were detected between group 1 and groups 4, 5, and 6, and between group 3 and group 6. Between groups 1 and 2, p = .059.

§Statistically significant differences were detected between group 1 and groups 4, 5, and 6, and between group 6 and all other groups.

available for IL-8 analysis. The IL-8 concentrations (picograms per milligram) in MEF1 ranged from 373 to 124,000 (mean 19,470, median 9,520). The IL-8 concentrations in MEF2 ranged from 440 to 903,700 (mean 79,075, median 15,274). The overall levels of LTB4 and IL-8 were not significantly different in MEF1 and MEF2 samples. The levels of these mediators were also not related to the age of the patient, number of episodes of previous otitis media (OM), duration of respiratory symptoms, or duration of OM symptoms prior to enrollment into the study. No obvious correlation existed between the LTB4 and IL-8 concentrations in the MEF. The Pearson correlation coefficients between log-transformed LTB4 and IL-8 concentrations in MEF1 for six microorganism groups (see Table 2 for groups) were from –.12 to .38 (p >.29).

LTB4 and IL-8 Versus Viral and Bacterial Infections. On the basis of the presence of viruses and bacteria, MEF1 samples were divided into six groups (Table 2). The levels of LTB4 and IL-8 related differently with the various pathogen groups.

The LTB4 levels in group 4 (bacterial infection), group 5, and group 6 (bacterial and viral infections) were statistically significantly higher than that in group 1 (no pathogen). The difference between LTB4 levels in group 2 (viral infection of the respiratory tract) and group 1 (no pathogen) was close to the significant level. No statistically significant difference was observed for LTB4 levels in group 1 and group 3 (virus but no bacteria in the MEF). On the other hand, the LTB4 level in group 6 was statistically significantly higher than that in group 3 (bacterial effect). Other group differences for LTB4 levels were not statistically significant. The IL-8 levels were not significantly different among groups 1, 2, and 3 (nonbacterial groups). That is, no virus-related effect was observed for IL-8 levels when bacteria were absent. However, the IL-8 levels were significantly higher in groups 4, 5, and 6 (bacterial groups) than in group 1 (no pathogen). In addition, the IL-8 levels in group 6 (both bacterial and viral infections) were significantly higher than in all five of the other groups. Groups 2, 3, 4, and 5 were not significantly different for IL-8 levels.

TABLE 3. LTB4 AND IL-8 CONCENTRATIONS IN MEF SPECIMEN FROM SUBJECTS WITH VIRUS IN ONE MEF SPECIMEN AND NO VIRUS IN CONTRALATERAL MEF SPECIMEN

	Leukotriene B4 (pg/mg)		*Interleukin-8 (pg/mg)*	
Patient	*No Virus*	*Virus*	*No Virus*	*Virus*
56	573	3,109	7,630	50,150
119	2,355	1,736	33,440	71,120
143	311	1,995	20,300	42,200
147	96	399	7,360	14,300
151	346	972	30,900	42,686

Respiratory syncytial virus was detected in 4 subjects, and influenza virus type B in 1 subject. All but 1 patient (No. 151) had same bacteria in both middle ear fluid (MEF) specimens.

To demonstrate the effect of viral infection on inflammation of the middle ear, subjects with MEF samples from one ear containing virus and contralateral MEF samples containing no virus were compared as shown in Table 3. The levels of LTB4 were higher in the MEFs containing virus than those containing no virus in all but 1 case (No. 119). For IL-8, all MEFs containing virus contained higher IL-8 levels than the MEFs on the contralateral side containing no virus.

LTB4 and IL-8 Versus Clinical and Bacteriologic Outcome. Higher levels of LTB4 in MEF1 were associated with bacteriologic failure 2 to 5 days after initiation of antibiotic treatment (p = .05, Table 4). Relationships between LTB4 and IL-8 levels in MEF1 and clinical outcome at 9 to 12 days (visit 3) and 1 month (visit 4) are shown in Table 5. At visit 4 (1 month), the recurrence rate of OM was directly cor-

TABLE 4. RELATIONSHIP OF INTERLEUKIN-8 AND LEUKOTRIENE B4 CONCENTRATIONS IN MIDDLE EAR FLUID SPECIMEN AT FIRST VISIT VERSUS CLINICAL OR BACTERIOLOGIC OUTCOME AT SECOND VISIT

	Leukotriene B4 (pg/mg)			*Interleukin-8 (pg/mg)*		
	N	*Median*	*Mean ± SE*	*N*	*Median*	*Mean ± SE*
Clinical outcome						
Improved	150	811	2,789 ± 547	122	9,234	18,659 ± 2,163
Failed	16	1,689	3,366 ± 1,627	11	22,142	29,658 ± 7,447
p		.35			.35	
Bacteriologic outcome						
Cured	91	1,114	3,242 ± 819	76	11,307	22,288 ± 2,962
Failed	18	2,995	5,684 ± 1,766	11	19,360	25,081 ± 6,547
p		.05			.67	

Second visit was 2 to 5 days after initiation of antibiotic treatment.

related with the levels of IL-8 in the MEF1 samples.

Associations were also evaluated between clinical or bacteriologic outcome at visit 2, or type of antibiotic used, and changes in LTB4 and IL-8 levels between MEF1 and MEF2 of the same patient (data not shown). Failed outcome and type of antibiotics were not related to increased or decreased levels of LTB4 and IL-8.

DISCUSSION

Our results point to the important role played by the mediators involved in PMN-induced inflammation in AOM. While the role of LTB4, a product released by PMNs, in the pathogenesis of OM has been extensively studied in clinical and experimental animal models of bacterial OM,[13,14,16,17] it has not been studied in viral OM. Furthermore, the role of IL-8, a potent chemotactic agent for PMNs, in either bacterial or viral OM has not been previously reported. Clinical investigations regarding the role of PMNs in the pathogenesis of AOM are important, since we have previously shown in animal models that PMNs are the major contributors to early inflammation in bacterial OM[12]; further studies may help develop strategies for controlling such inflammation.

Leukotriene B4 is primarily synthesized by PMNs as a product of arachidonic acid metabolism via the lipoxygenase pathway.[18] A study of experimental pneumococcal OM in chinchillas has suggested that arachidonic acid products, including LTs, in the middle ear derive from inflammatory cells, since these products appear after influx of inflammatory cells into the area.[17] Leukotriene B4 promotes chemotaxis of PMNs and eosinophils, increases vascular permeability, and causes hyperalgesia. In animal models of AOM, the concentrations of LTB4, among other LTs, in the MEF have been shown to correlate well with the degree of inflammation in the middle ear.[13] In subjects with chronic OM, higher levels of LTB4 are found in bacterial culture–positive MEFs than in sterile MEFs.[16] Data from our present study of AOM show higher LTB4 levels in the MEF compared to those reported by Brodsky et al,[16] who measured LTB4 concentrations in MEFs from subjects with chronic OM effusions. These observations suggest that the concentrations of LTB4 may be related directly to the acuteness of the middle ear disease, and to the degree of inflammation.

The role of PMNs in virus-induced inflammation

TABLE 5. RELATIONSHIP OF LEUKOTRIENE B4 AND INTERLEUKIN-8 CONCENTRATIONS IN FIRST MIDDLE EAR FLUID SPECIMEN VERSUS CLINICAL COURSE AT THIRD VISIT (DAYS 9 TO 12; END OF TREATMENT) OR AT ONE-MONTH VISIT

	Leukotriene B4 (pg/mg)			*Interleukin-8 (pg/mg)*		
	N	*Median*	*Mean ± SE*	*N*	*Median*	*Mean ± SE*
At visit 3						
Good response	106	749	2,938 ± 732	91	7,850	17,675 ± 2,547
Persistent otitis*	56	1,146	2,897 ± 659	39	13,224	22,940 ± 3,871
p		.18			.27	
At 1-month visit						
No recurrence	97	774	2,036 ± 306	80	8,331	16,958 ± 2,383
Recurrence†	46	1,031	3,805 ± 1,538	33	14,100	28,802 ± 5,498
p		.54			.04	

*Clinical and/or bacteriologic failure observed at either visit 2 or visit 3.

†Presence of acute otitis media at visit 4, approximately 1 month after enrollment.

of the middle ear is poorly understood. However, there is increasing evidence that PMNs can also play a major role in inflammation-associated viral infection. Previous clinical studies have shown that respiratory viral infection is associated with the presence of LTs, including LTB4, in respiratory secretions.[19,20] In vitro studies have also shown that RSV-exposed macrophages and PMNs release leukotrienes.[21,22] In the current study, there was a trend for viral infection of the respiratory tract in vivo to be associated with the production of LTB4 during AOM. In the overall analysis among groups of patients with different sites and types of infections (Table 2), LTB4 levels were higher in MEFs from patients with viral infection of the respiratory tract (group 2) compared to those associated with no identifiable pathogen (group 1). In addition, in 5 of 6 individual patients with disparate virologic data between left and right MEFs (Table 3), LTB4 levels were higher in MEFs containing viruses. It is likely that proinflammatory cytokines and other soluble mediators produced locally (eg, in the respiratory tract or the middle ear) during the acute phase of viral illness can prime the PMNs to release LTB4.

Since LTB4 may be a key inflammatory product that sustains inflammation, the effect of antibiotic treatment on subsequent levels of LTB4 was also evaluated. Treatment with antibiotics did not result in a significant reduction in the levels of LTB4 at visit 2 (2 to 5 days). This observation is in keeping with our previously reported observation that levels of histamine, another potent inflammatory mediator, were also not significantly reduced at the same follow-up visit.[23] These observations suggest that treatment of AOM may require adjunctive therapeutic agents such as antiviral and/or anti-inflammatory agents that reduce the levels of inflammatory mediators or enhance the eradication of bacteria.

Since the presence of LTB4 indicated the presence of PMNs in the middle ear, our detection of IL-8, a potent cytokine that promotes the migration of PMNs, is also important. In the present study, the levels of IL-8 in MEFs correlated with several aspects of OM. Higher levels of IL-8 were found in subjects with combined viral and bacterial infection of the middle ear (group 6) than in all other groups. Furthermore, IL-8 levels were higher in MEFs containing bacteria and virus, compared to those containing bacteria alone (Table 2). This observation suggests that viruses contribute synergistically to production of IL-8 in the middle ear during bacterial infection. There have been numerous reported cell sources of IL-8, including monocytes, macrophages, fibroblasts, endothelial cells, epithelial cells, and lymphocytes.[24] The sources of IL-8 in the middle ear are most likely to be epithelial cells and fibroblasts that have been exposed to bacteria and viruses in the earlier stage, and macrophages in the later stage. It is interesting that higher levels of IL-8 in the initial MEF were related to a higher recurrence rate of OM within a month of follow-up. The reason for this is not clear. In our animal models,[12] inhibition of PMN migration to the middle ear is associated with a reduced degree of inflammation as shown by histology. It is possible that increased PMN migration into the middle ear leads to an increased and sustained tissue injury, and injured tissue may be more susceptible to recurrent bacterial invasion.

In summary, our study suggests that PMNs may be important in the bacteriologic and clinical response in AOM. This study highlights the need for adjunctive therapies that control the PMN-mediated inflammatory response.

ACKNOWLEDGMENT — We thank Dr Constance Baldwin for reviewing the manuscript and for editorial assistance.

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Ann Otol Rhinol Laryngol 105:1996

HIGH-SPEED IMAGING OF VOCAL FOLD VIBRATIONS AND LARYNX MOVEMENTS WITHIN VOCALIZATIONS OF DIFFERENT VOWELS

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Theoretic investigations of the "source-filter" model have indicated a pronounced acoustic interaction of glottal source and vocal tract. Empirical investigations of formant pattern variations apart from changes in vowel identity have demonstrated a direct relationship between the fundamental frequency and the patterns. As a consequence of both findings, independence of phonation and articulation may be limited in the speech process. Within the present study, possible interdependence of phonation and phoneme was investigated: vocal fold vibrations and larynx position for vocalizations of different vowels in a healthy man and woman were examined by high-speed light-intensified digital imaging. We found 1) different movements of the vocal folds for vocalizations of different vowel identities within one speaker and at similar fundamental frequency, and 2) constant larynx position within vocalization of one vowel identity, but different positions for vocalizations of different vowel identities. A possible relationship between the vocal fold vibrations and the phoneme is discussed.

KEY WORDS — high-speed imaging, larynx, vocal fold vibration, vowel.

INTRODUCTION

In both the acoustic and the physiologic theory of voiced speech sounds, phonation and articulation are generally regarded as independent of each other. Phonation produces the sound with its general characteristics, including fundamental frequency (F0), and articulation transforms the sound into a phoneme by the resonance pattern (formant pattern) of the vocal tract. Yet, two findings indicate that such independence may be limited in practice: the glottal source and vocal tract acoustically interact, and the lower formants are dependent on the F0 of vocalization.

Theoretic analysis and simulation of speech based on models of speech production show a pronounced interaction between vocal tract shape and glottal volume flow.[1-4]

Analysis of sung vowels indicates a relationship among all formants and F0.[5-7] In vowel synthesis, the F1 of all vowels, and the F1 and F2 of back vowels, have to be altered with alterations of F0 in order to maintain the vowel identity.[8,9] A similar observation was made concerning natural vowels, produced in monotone and in isolation (apart from singing or shouting), since the frequency levels of the F1 of all vowels, and of the F1 and F2 of back vowels, are found to relate to F0.[10-12]

In consequence, a double relationship of glottal source and vocal tract is indicated: the glottal source is acoustically influenced by the vocal tract shape, and the formant pattern of a vowel sound is related to F0, ie, to the frequency of the periodic vocal fold movements. In terms of speech production theory, the source is influenced by the filter, and the filter configuration is adjusted according to one source characteristic, the F0. Therefore, the question arises whether, in real speech, a physiologic interaction of phonation and articulation can be observed.

As a first step toward experimental evidence, we have followed a general approach: Does the vocal fold vibration pattern differ for different vowel identities being produced?

Direct investigation of the vocal fold vibrations in normal speech is no simple matter because of the severe difficulty of portraying the vocal fold vibrations under natural conditions of speech, and of relating the images to acoustic features. None of the common "direct" methods, such as electromyography,[13] electroglottography (EGG),[14,15] or stroboscopy,[16] could provide an unambiguous illustration of these speech movements at a differentiated level. However, with the new technique of high-speed light-intensified digital image recording, observation of the vocal fold movements in real speech became possible. This article describes our experimental approach, and reports two observations indicating a possible relationship between vocal fold movements

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TABLE 1. INVESTIGATED RECORDINGS

Vowels	*Vocalization Type**	*Sex of Speaker*	*No. of Recordings†*
/a/	1	M	4
/a/	1	F	3
/e/	1	M	2
/e/	1	F	1
/i/	1	M	2
/i/	1	F	1
/o/	1	M	2
/o/	1	F	2
/u/	1	M	2
/u/	1	F	3
/a/, /e/, /i/, /o/, /u/	2	F	6
/a/, /e/, /i/, /o/, /u/	2 (sung)	F	1

*1 — Vowel produced in isolation, and sustained at given pitch, 2 — all five vowels produced in series within one vocalization, at constant fundamental frequency.

†Recordings with sufficient optical and acoustic quality, which were retained for analysis.

and vowel identity.

METHOD

The vocalizations of two healthy adult speakers, one woman and one man, were investigated by the technique of high-speed light-intensified digital image recording[17] with transnasal fiberoscopy of the larynx, with the tip of the fiberscope in an endolaryngeal supraglottic position.

Vowels and Speakers. The five German vowels /u/, /o/, /a/, /e/, and /i/, vocalized by a woman (29 years old) and a man (34 years old), were investigated.

Vocalization Types. Two different types of vocalizations were studied: either a vowel was produced in isolation, and sustained at a given F0 (vocalization type 1); or all five vowels were produced in a series within one vocalization, at constant F0 (vocalization type 2). Different orders of the five vowels within one vocalization were investigated.

At the beginning, we intended only to observe the vocal fold vibrations within vocalization type 1. But when we observed the vibrations of the man, studied first, we could see clear adjusting movements of the entire larynx (approximation of arytenoids, elevation of the larynx, prephonatory adduction of vocal folds) in relation to a given vowel identity. Since, often, the position of the laryngoscope could not be held constant between two different vocalizations, we decided to add the second vocalization type for the examination of the woman. Therefore, vocalization type 2 is only investigated for the vowels of the woman.

In analyzing vocalizations of type 1, the movements within one period of the vocal fold oscillation can be investigated, and in analyzing vocalizations of type 2, the aforementioned adjustments of the larynx can be considered.

Recordings of Images and Sounds. Approximately 2.5 seconds of the acoustic and EGG signal were recorded in parallel, with a sampling frequency of 10 kHz. Sound pressure level was measured in decibels (A-weighted) at a distance of 1 m (Bruel & Kjaer 2203). Transnasal fiberscopy of the larynx (Olympus P3) was applied to record the relevant 2.5 seconds of the vocal fold vibrations with a high-speed light-intensified digital camera (Kodak EktaPro EM Motion), with a sampling frequency of 3,000 images per second (for technical details, see Hess and Gross[17]). The digitally recorded images were transferred to videotape off-line, image by image.

As a result of this procedure, we obtained three recordings that correlate in the time domain, the acoustic signal, the images of the vocal fold vibrations, and the EGG curves. Table 1 gives the numbers of recordings that yielded sufficient quality of images and sound, and that were retained after the vocalization sessions for further analysis.

Analysis of Vocalizations of Type 1. For each of the recordings, first, optical and acoustic quality was checked. Second, the constancy of the vocal fold movements over the entire duration of a vowel sound was examined: the investigators compared the opening and closing gestures of the vocal folds, period by period, using the slow-motion mode of the video recorder, and examined whether the entire movement within one period was similar to or different from the movement of the subsequent periods. If the investigators judged the periodic movements of a vocalization to be similar, one single period of the vocal fold oscillation in the middle of a vocalization was determined and printed out, image by image. Thus, series of images showing one oscillation period of the vocal folds for a given vocalization were produced.

In parallel, EGG curves were examined, and the pitch periods of EGG and of images were compared.

Analysis of Vocalizations of Type 2. For each of the recordings, again, optical and acoustic quality was checked first. Second, the investigators determined which time fragments of the entire vocalization related clearly to one vowel identity, and which related clearly to transitions from one vowel to another. The transitions were acoustically easy to recognize, since short interruptions of the phonation between the vowels were apparent. The selection of the time fragments was carried out on the basis of acoustic judgment and analysis of the sound spectrum. Third, for each of the time fragments deter-

TABLE 2. OPEN QUOTIENT FOR DIFFERENT VOWELS OF MALE SUBJECT WITHIN ONE PERIOD OF VOCALIZATION

Vowel	*Ratio of "Opened" to "Closed"*	*Open Quotient*	*No. of Images per Period*	*F0 (Hz)*	*Sound Pressure Level in dB(A)*
/a/	16:12 = 1.33	0.57	28	107	70
/a/	17:12 = 1.42	0.59	29	104	72
	(16:13) = (1.23)	(0.55)			
/a/	18:10 = 1.8	0.64	28	107	68
/a/*	19:9 = 2.11	0.68	28	107	70
/e/	18:10 = 1.8	0.64	28	107	66
	(17:11) = (1.55)	(0.61)			
/e/	19:13 = 1.46	0.59	32	94	66
	(20:12) = (1.67)	(0.63)			
/o/	16:12 = 1.33	0.57	28	107	64
/o/	15:13 = 1.15	0.54	28	107	66
/i/	15:13 = 1.15	0.54	28	107	61
/i/	12:16 = 0.75	0.43	28	107	60
	(11:17) = (0.65)	(0.39)			
/u/*	12:16 = 0.75	0.43	28	107	65
	(11:17) = (0.65)	(0.39)			
/u/	10:19 = 0.53	0.35	29	104	66
	(11:18) = (0.61)	(0.38)			

In column 2, if determination was ambiguous, second ratio is given in parenthesis.
F0 — fundamental frequency of period.
*Compare Fig 1.

mined, several images at different points in time were selected and printed out.

Thus, series of images were produced showing the vertical laryngeal placement and endolaryngeal adjustments for one vowel, as mentioned above, and showing the laryngeal adjustment between two different vowels.

RESULTS

Having analyzed all the empirical material, we obtained four main findings.

Methodological Problem of Small Number and Inconsistent Quality of Recordings. As may be expected, there are several problems inherent in the method of investigating the relationship between vocal fold behavior and vowel sounds. On one hand, the investigation itself is inconvenient for the individuals examined. Local anesthesia has to be repeated several times. The placement of the fiberscope is critical, and many recordings were therefore of impaired quality and had to be repeated several times. On the other hand, the sampling frequency for the images is problematic. If the sampling frequency is >3 kHz, the image size becomes too small for analysis, because it decreases with increasing sampling frequency.[17] But we consider a sampling frequency of 3 kHz as already too low for the analysis of the periodic vocal fold movements of a woman.

In consequence, despite extensive recording time and expensive material, variable recording quality and a limited frequency range of F0 resulted. We therefore decided to limit our examination to two basic observations concerning the periodic movement of the vocal folds and the larynx position. Because of the differences in the recorded vocalization types between the man and woman, and because of the higher F0 for vocalizations of type 1 by the woman, the vocalizations of the man were analyzed with regard to the relationship between the vocal fold movement within one oscillation period and the vowel identity; the vocalizations of the woman were analyzed with regard to the relationship between the larynx position and the vowel identity.

Tendency Toward Vowel-Dependent Movements of Vocal Folds. The authors judged the form of a periodic movement of the vocal folds to be similar within one vocalization, in the sense that a comparison of different periods within one vocalization shows similar movement processes of opening and closing gestures. Conversely, there is no general similarity between the different vocalizations of the man, despite the fact that the frequency levels of F0 are similar. In consequence, the question arises as to whether these differences relate to different phonation properties aside from the phoneme identity, or whether they are related to the vowel itself. To answer that question, a first step toward quantification is needed. Thus, we decided to examine the

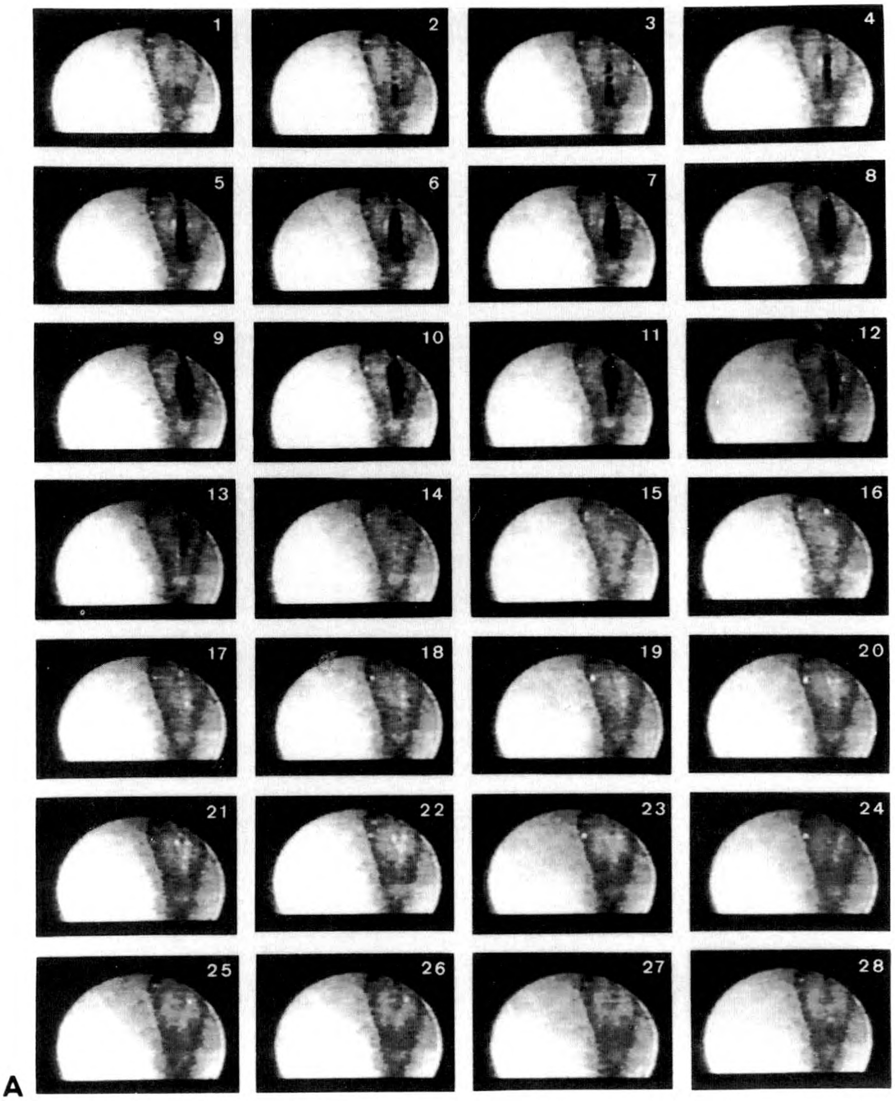

Fig 1. Two periods of two different vowels — A) vowel /u/, and B) vowel /a/ — in sense of opening and closing gesture of vocal folds. Both vowels were produced by same male subject. Fundamental frequency is 107 Hz; corresponding image number for one period is 28. Comparison represents example of different vocal fold movements within vocalizations of same speaker. For /u/, vocal folds are held "closed" longer than "opened" within period, but inverse ratio is found for /a/ (for numeric values, see Table 2). (Continued on next page.)

relationship between the "opened" and the "closed" glottis within one period, in the sense of establishing a numeric ratio of images, to determine the open quotient (OQ; see formula below). The glottis was regarded as "opened" if the vocal folds did not touch each other over more than half their length. Otherwise, the glottis was regarded as "closed."

The formula applied is OQ = [N(o)]/[N(p)], where N(o) is the number of images within the period showing an "open" glottis, and N(p) is the number of images of the entire period.

By calculating the OQ for those recordings of the man that were sufficient in regard to optical and acoustic quality, we obtained numeric results as given in Table 2. These values indicate a tendency toward a vowel-dependent OQ. For the vowels /a/ and /e/, the "opened" phase of the oscillation period is longer than the "closed" phase; the same holds true for /o/, but less markedly; and inversely, for /u/, the "closed" phase is longer than the "opened" one. For /i/, the variations are large, and the values lie somewhere between those of /o/ and /u/. The most obvious difference is found for /u/ compared with /a/ and /e/. Figure 1 gives a comparison of /u/ and /a/ illustrating this difference.

We also examined the vowels produced by the woman, and here we obtained ambiguous results. The range of F0 was ≥272 Hz, and the corresponding length of a period was ≤11 images. The determination of the OQ was not as clear as in the vowels of the man, mostly because the resolution of the period was much too low. No evidence was found for an unambiguous difference between /u/ and /a/. We could not find any clear indications as to whether this finding was a consequence of an insufficient sampling rate for the images, or whether the differences in the vocal fold movements observed in the case of the man were only accidentally related to the vowel identity.

Incongruency of EGG Curves and Images of Vocal Fold Vibrations. Having noted such differ-

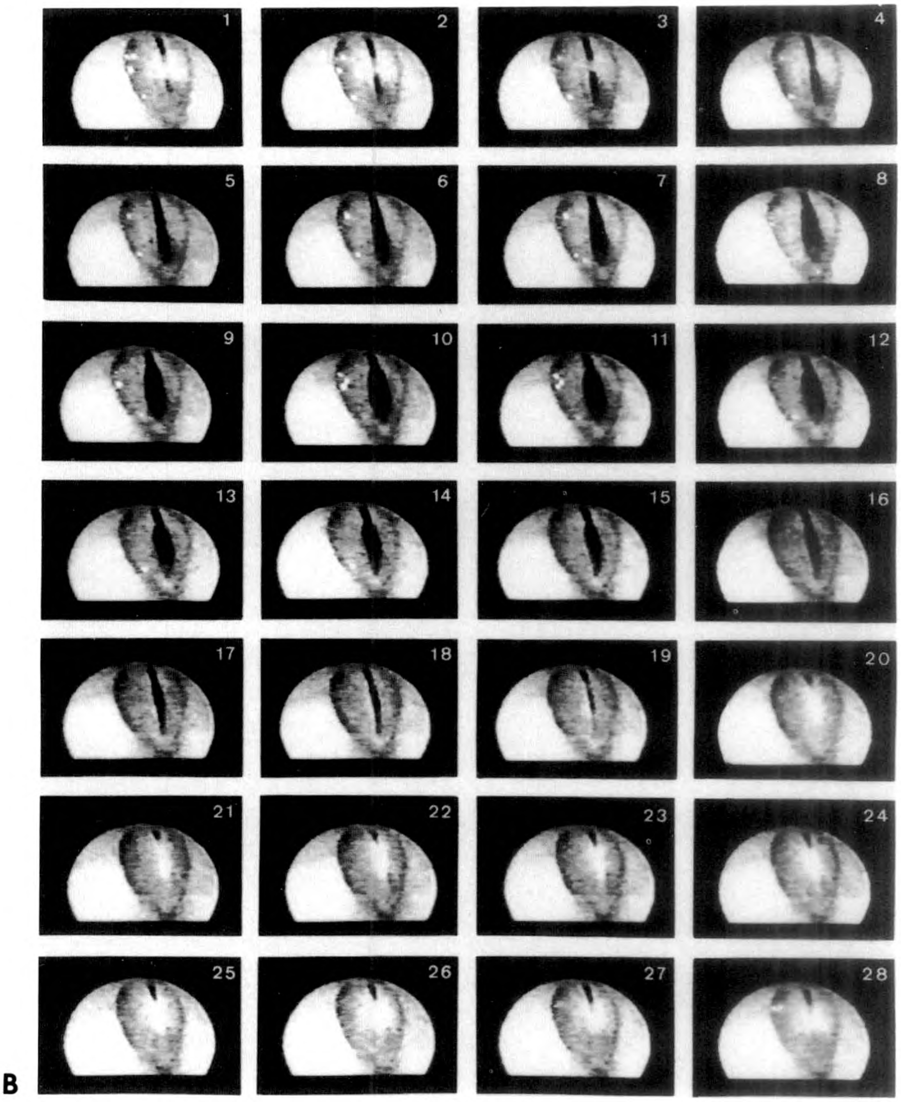

Fig 1, continued. Vowel /a/.

ences in the vocal fold behavior of the man within one oscillation period on the basis of the high-speed recorded images, we tried to find parallel differences in the related EGG curves. We estimated closed quotients from the EGG signals for the time fragments that related to the high-speed recorded images (bandwidth of the upper peak 30% below period to period distance was measured for the closed phase), and then derived OQs. A comparison of the vocalizations with different vowel identities showed similar values for the four vowels /o/, /a/, /e/, and /i/. Only the vocalizations of /u/ showed markedly lower values than the other vocalizations. In consequence, concerning the OQ, no simple parallelism of high-speed images and EGG curves was found. Conversely, the F0 of images and EGG coincided for all vocalizations investigated.

Different Larynx Positions for Different Vowels. When the vocalizations of the woman (vocalization type 2) were examined, all of the six normal vocalized vowel series showed a clear movement of the larynx position between two vowels. When producing a vowel, the woman held the larynx position constant, but when she changed from one vowel to another, she altered that position, in the sense that a rearrangement of the entire larynx structure was observed (approximation of arytenoids, elevation of larynx, prephonatory adduction of vocal folds). Figure 2 gives two examples of such vowel-dependent larynx placements.

Only for the sung vocalization did we have difficulty determining distinct larynx positions that could clearly be related to the different vowels.

DISCUSSION

Our initial question was whether the vocal fold vibration pattern differs for different vowel identities. The results obtained do not allow for any final conclusion, but two observations indicate a possible interdependence of vocal fold vibrations and vowel

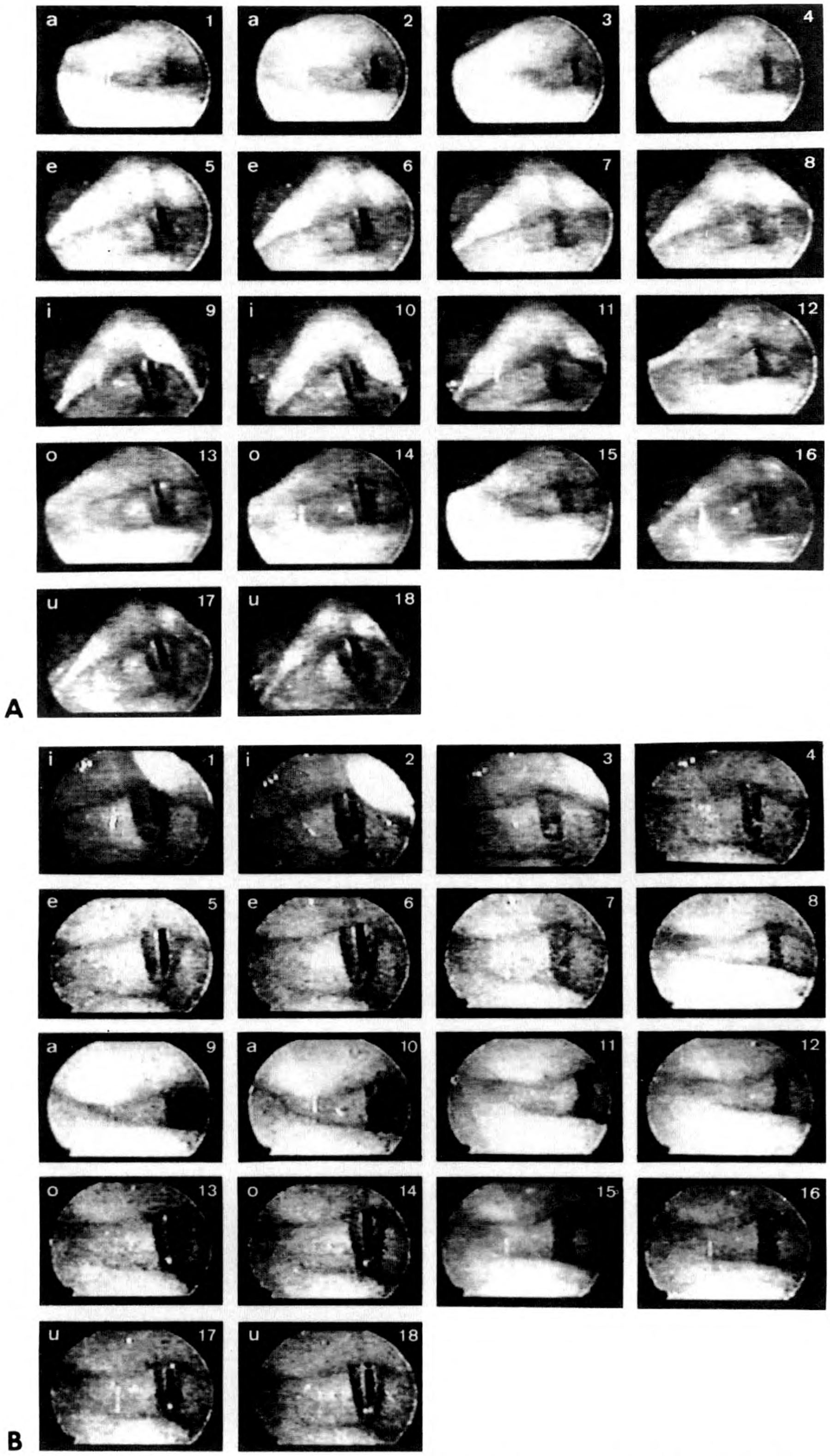

Fig 2. Two examples of larynx movements within two vocalizations of five vowels in series. Both vowel series were produced by same female subject. A) Vocalization of five vowels in series. For each vowel identity, two positions of larynx at different points in time are given first, and vowel identity is assigned on left; between these images, two positions of larynx at different points in transition time between two subsequent vowel identities are given (eg, images 1 and 2 for /a/, images 3 and 4 as transition to images 5 and 6 for /e/, images 7 and 8 as transition to images 9 and 10 for /i/, etc). B) Vocalization of same five vowels as in A, but with vowels in different order. When vowel was produced, larynx was held in constant position (compare two images on left of any line, marked with vowel identity); but when vocalization proceeded from one vowel to another, larynx obviously changed its position (compare two images on right side of any line, representing transition from preceding to following vowel identity within same vocalization series). In all images, epiglottis is seen at bottom, and arytenoids during adduction form upside-down V-shape on top of images. Different adduction distances for false vocal folds are observed. Furthermore, different distances between tip of fiberscope and vocal folds indicate laryngeal vertical placement changes.

identity. First, within the normal vocalizations of one speaker (chest register) and at similar F0, different movements of the vocal folds can be observed for different vocalizations, and the differences we observed tended to be related to the vowel identities. Second, placement movements of the larynx that are clearly related to vowel identity can be observed within one vocalization of different vowels. These placements can be interpreted as a part of the adjustment of the vocal tract, but, conversely, they can also indicate a regulation of the vocal folds, since they include adjustments of the arytenoids, as mentioned above.

The results are preliminary, since they are based on two single case studies, but they show that no simple evidence is given for a uniform vocal fold vibration pattern in normal speech.

A possible relationship between vocal fold movements and vowel identity, as indicated in our study, may be mainly explained as a consequence of either 1) individual phonation characteristics of the subjects examined, in the sense that the tension of the vocal folds is not held uniform for different phonemes in speech, or 2) the acoustic influence of the vocal tract shape. Obviously, the observed differences of the vocal fold movements could also mirror both influences in combination.

However, further investigations should also consider a vowel dependence of phonation apart from individual phonation characteristics and from acoustic interaction of glottal source and vocal tract, even if such dependence may appear as unlikely within the traditional understanding of phonation and articulation. As mentioned above, recent findings indicate that the parallelism between vowel identity and characteristic formant pattern is in contrast to the F0 dependence of the pattern. In addition, number alterations of the formants ("one-formant" back vowels and "two-formant" front vowels) can regularly be found in real speech, particularly in women and children (for example, see Maurer and Landis[12]). Moreover, a physiologic investigation of possible articulatory variations apart from changes in vowel identity indicated that there is only a partial and vague correlation between articulator positions and the vowel sound being produced.[18] In consequence, there is no simple parallelism between one vocal tract configuration and one vowel identity, and the physiological process of articulation is not satisfactorily understood. Hence, theoretic assumptions concerning a possible interdependence of articulation and phonation have to be cautiously considered, since both physiologic processes are closely linked to each other.

On the basis of these considerations, we conclude that the question of the physiologic role and interaction of phonation and articulation has to be investigated in more detail. One of the most important topics concerns the relationship between observable vocal fold movements and their acoustic correlates, aside from pitch alterations and aside from speech disorders. For such an investigation, the experimental technique has to be refined, in order to examine a larger body of material.

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DISTORTION PRODUCT OTOACOUSTIC EMISSION TEST OF SENSORINEURAL HEARING LOSS IN HUMANS: COMPARISON OF UNEQUAL- AND EQUAL-LEVEL STIMULI

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Distortion product otoacoustic emissions (DPOEs) at the frequency of 2f1 – f2 (f1 < f2) were measured in 77 human adult ears with normal hearing or sensorineural hearing loss. The purpose of this study was to compare the performances of DPOE tests conducted with two sets of stimuli: 1) L1 = 65, L2 = 50 dB sound pressure level (SPL) re 20 μPa ("65/50"), and 2) L1 = L2 = 65 dB SPL ("65/65"). Half-octave DPOE root-mean-square levels at 1,000, 2,000, 4,000, and 6,000 Hz were computed from the initial DPOEs measured at 0.25-octave intervals. Correlation coefficient and decision-theory analyses were applied to evaluate the DPOE test performance. For both stimuli, DPOE level exhibited significant correlation with pure tone hearing threshold. When the criterion DPOE level distinguishing normal from impaired hearing was adjusted, the curves of sensitivity and specificity crossed, and the values at the crossing were higher than 80% at frequencies of 2,000 to 6,000 Hz for both stimuli. The area under the receiver operating characteristic (ROC) curve, which provides an overall evaluation of the test performance independent of the criterion DPOE level, was .90 or higher at 2,000 to 6,000 Hz for both stimuli. At 2,000 and 4,000 Hz, all measures of test performance were higher for the 65/50 stimulus than the 65/65 stimulus: area under the ROC curve (.96 to .97 versus .90 to .91, statistically significant, $p < .001$, Wilcoxon test), sensitivity/specificity (90% to 93% versus 80% to 85%), and correlation coefficient (.78 to .87 versus .66 to .79). At 1,000 and 6,000 Hz, the performances of the DPOE tests were similar for the two stimuli. These results support the conclusion that a DPOE test with L1 = 65 and L2 = 50 dB SPL provides a better performance than that with L1 = L2 = 65 dB SPL and recommend the use of stimuli with L1 being higher than L2 by about 15 dB. These results also support a growing view that 2f1 – f2 DPOEs can be utilized clinically as a reliable method of testing human sensorineural hearing loss.

KEY WORDS — audition, cochlea, ear, predictive efficiency, receiver operating characteristic, sensitivity, specificity.

INTRODUCTION

Distortion product otoacoustic emissions (DPOEs) were recorded acoustically in the ear canal in both humans[1] and animals[2,3] many years ago. It is generally accepted that active, nonlinear biomechanical properties of the cochlea are responsible for generating DPOEs. By virtue of their noninvasiveness and frequency specificity, DPOEs are being developed into important laboratory and clinical methods for monitoring the functional status of the cochlea. The 2f1 – f2 component is most prominent among DPOEs for two primary tones having frequencies of f1 and f2 (f1 < f2), and hence is most commonly employed in studies of DPOEs.

Stimulus parameters, such as the frequency ratio f2/f1, the levels of the primary tones L1 and L2, and the level difference L1 – L2, influence DPOEs. It has become evident that the DPOE level varies with the frequencies of the primary tones. The dependence of DPOE level on the frequency ratio of the two primaries has been systematically studied.[4,5] These studies found that an f2/f1 ratio of about 1.22 was generally optimal to produce the highest DPOE level in normal human ears, although slight variation was present for different frequencies, stimulus levels, and subjects. Input-output functions derived in many investigations[6,7] have confirmed that the DPOE level is affected by the primary-tone level. Although the primary levels were varied in these studies, they were mostly equal levels, ie, L1 = L2.

The level difference L1 – L2 has been less systematically investigated in studies of human DPOEs than the f2/f1 ratio. Experiments in animals[8,9] observed that the primary tones were most effective when L1 was 5 to 15 dB higher than L2. The findings of psychoacoustic studies[10] agreed with this. In human ears with normal hearing, Gaskill and Brown[5] and Hauser and Probst[11] observed that stimuli with L1

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Presented at the meeting of the Association for Research in Otolaryngology, St Petersburg Beach, Florida, February 5-9, 1995.

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being higher than L2 by 5 to 20 dB produced maximum DPOE levels. While experimentally altering the endocochlear potential in animals, Mills et al[12] made an important finding that the amount of reduction in DPOE level produced by cochlear impairment was the greatest when L1 was higher than L2 by about 10 to 15 dB. They recommended the use of DPOEs with L1 about 10 dB above L2 for monitoring cochlear function. No information was available for *human* ears, until recently,[13] about a possible differential effect of cochlear impairment on DPOEs for equal versus unequal levels of the primary tones. For testing human sensorineural hearing loss, one may predict from the animal results of Mills et al that a DPOE test with L1 − L2 = 15 dB should be more effective than that with L1 = L2. The present study was specifically designed to examine this prediction. Accordingly, we conducted a study evaluating the performances of DPOE tests with the two types of stimuli for detecting human sensorineural hearing loss. The test performance was evaluated in terms of the sensitivity, specificity, predictive efficiency,[14] and area under the receiver operating characteristic (ROC) curve.[15,16] This study was presented at the 1995 meeting of the Association for Research in Otolaryngology.[17]

MATERIAL AND METHODS

Subjects. Data were collected from 77 ears of 44 human adult subjects 18 to 75 years old. Subjects, selected without targeting sex or race, had good general health by history, and were taking no medications on a regular basis. All ears presented a normal (type A) tympanogram and air-bone gaps of less than 10 dB. Voluntary consent was acquired for every subject.

We defined normal hearing threshold to be 20 dB hearing level (HL) (re: ANSI, 1969) or better at each of the standard audiometric frequencies (0.25, 0.5, 1, 2, 4, and 8 kHz; and 3 and 6 kHz when tested) and an average of these thresholds of 10 dB HL or better. Of the 77 ears, 32 ears from 20 subjects showed normal hearing. Hearing loss was defined to be 25 dB HL or worse at any frequency. Forty-five ears from 28 subjects exhibited sensorineural hearing loss. The amount of hearing loss in this group ranged from 25 to 95 dB. Of the 45 ears, 18, 18, 6, and 3 ears had mild (25 to 40 dB), moderate (45 to 60 dB), severe (65 to 80 dB), and profound (≥85 dB) hearing loss, respectively, according to the maximum amount of hearing loss across frequency. We did not specifically target any particular audiometric pattern of sensorineural hearing loss. The audiograms of the hearing-impaired ears of this study were of the following types: 1) with a gradual slope (≤15 dB/octave) falling toward the high frequencies (9 ears); 2) with a steep slope (≥20 dB/octave) falling toward the high frequencies (19 ears); 3) with a trough, often centered at 3 to 4 kHz (15 ears); and 4) irregular (2 ears). This sample excluded retrocochlear hearing loss as judged by audiologists and otolaryngologists.

Seven of the subjects had normal hearing in one ear and hearing loss in the other ear. When the normal-hearing ears were analyzed as a group, the age of the subjects was restricted at 18 to 35 years in order to minimize the influence of age. We required the normal-hearing ears to have a history negative for long-term noise exposure, ototoxic drug administration, ear infections, and familial hearing impairment. Twenty-five ears fell into this group.

Apparatus and Stimulus Parameters. The DPOE measurement system, including the stimulus generation and response detection equipment, is the same as those described in previous reports from our laboratory.[16,18] Briefly, this system consisted of an IBM PC–compatible computer, an Ariel DSP-16 board containing two-channel 16-bit digital-to-analog and analog-to-digital converters installed in the computer, two ER-2 earphones, and an ER-10B low-noise microphone system (including an amplifier that provides 40 dB gain). The data were collected by means of the "2f1 – f2 DPOE level versus frequency" paradigm. The f2/f1 ratio was kept at approximately 1.2 (varying from 1.18 to 1.23). The frequencies f1 and f2 were varied at 0.25-octave steps. Two sets of primary tone levels were used: 1) L1 = 65 and L2 = 50 dB sound pressure level (SPL) re 20 micropascals (referred to as "65/50") and 2) L1 = L2 = 65 dB SPL ("65/65").

Procedure and Data Treatment. Measurements were made with subjects seated inside a sound-attenuated chamber (Industrial Acoustics Co). Subjects were instructed to remain as quiet as possible during the test periods. Calibration of the acoustic stimulus level was performed as described previously.[16,18] One complete series of measurements lasted about 15 minutes per ear.

To smooth out peaks and troughs of DPOE level versus frequency, we converted the original DPOEs obtained at 0.25-octave intervals into half-octave root-mean-square (RMS) DPOE levels. This procedure, the same as described by Smurzynski et al,[19] is illustrated in Fig 1 with results from one normal-hearing ear. The DPOE levels originally measured at 0.25-octave intervals are shown in the left column of Fig 1, and the half-octave RMS DPOE levels derived from the original data, in the right column. The top two rows correspond to the two different stimulus

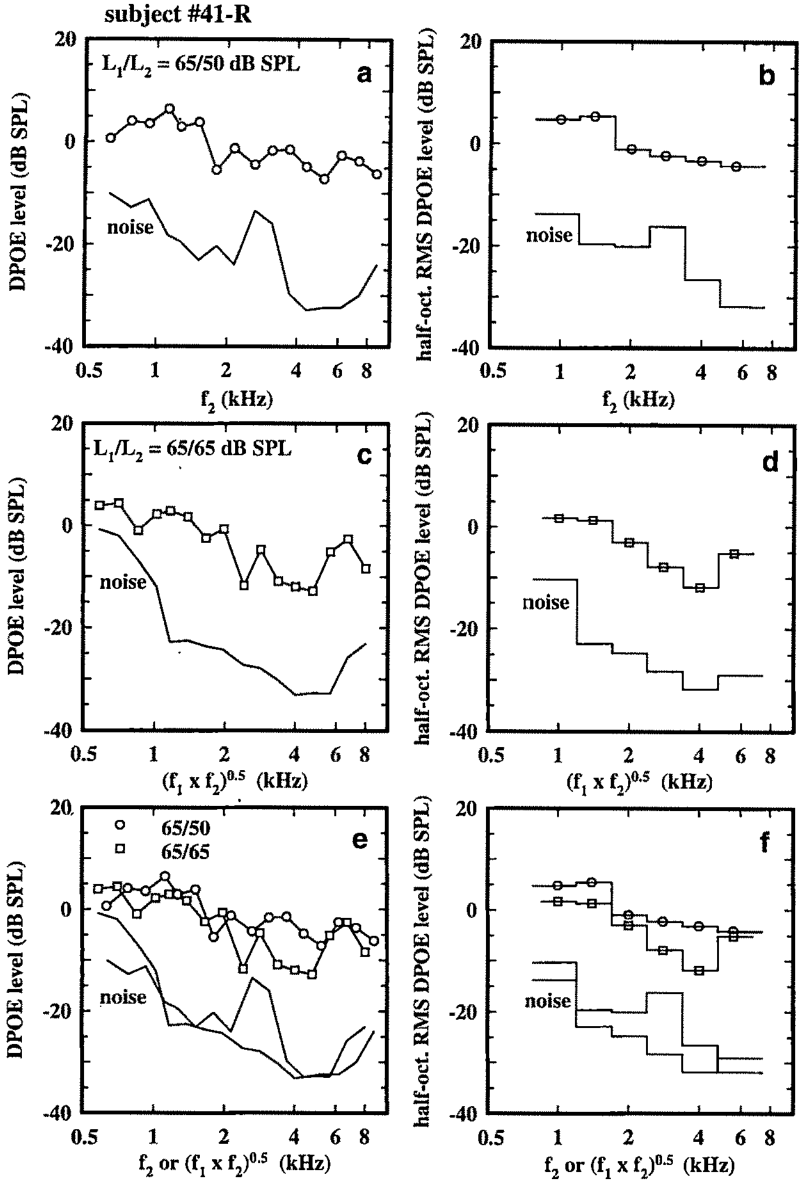

Fig 1. Distortion product otoacoustic emissions (DPOEs) from adult human ear with normal hearing. Top row — DPOE level versus f2 for stimuli with L1 = 65 and L2 = 50 dB sound pressure level (SPL). Middle row — DPOE level versus $(f1 \times f2)^{0.5}$ for L1 = L2 = 65 dB SPL. Bottom row — comparison of data in above two rows. Left column — raw data of DPOE level measured at 0.25-octave intervals. Right column — half-octave root-mean-square (RMS) DPOE level derived from raw data in left column.

conditions, and the bottom row, to a comparison of the above two. The example illustrates that the peaks and troughs of the original data are smoothed in the right column. Values of half-octave RMS DPOE levels at the standard frequencies, ie, 1,000, 2,000, 4,000, and 6,000 Hz, were interpolated from adjoining data within 0.25 octave of a standard frequency.

A number of studies[2,20-23] have suggested that the 2f1 – f2 DPOE is generated in an overlap region of the two primary frequencies along the cochlea. Considering the cochlear traveling wave envelopes associated with closely spaced f1 and f2 frequencies (eg, Figure 1 of Kim et al[16]), we anticipate that when L1 is equal to L2, the maximum overlap of the two frequency envelopes is located between the f1 and f2 places, and that when L1 is higher than L2 by 10 to 15 dB, the maximum overlap is close to the f2 place. From this reasoning, we paired the DPOE level with pure tone threshold at a frequency of f2 when L1 – L2 = 15 dB, whereas we paired the former with pure tone threshold at a frequency of $(f1 \times f2)^{0.5}$ when L1 = L2. From the same reasoning, we plotted DPOE level versus f2 for the 65/50 condition (Fig 1, top row), while plotting DPOE level versus $(f1 \times f2)^{0.5}$ for the 65/65 condition (Fig 1, middle row). In this context, for a particular frequency of pure tone threshold under consideration, the value of f2 under the 65/65 condition was slightly higher (by a factor of 1.095) than that of the 65/50 counterpart. This difference was small, less than 10%, in this study where f2/f1 was 1.2.

The analysis was made at frequencies of 6,000, 4,000, 2,000, and 1,000 Hz. Data for frequencies lower than 1,000 Hz and those for 8,000 Hz were not

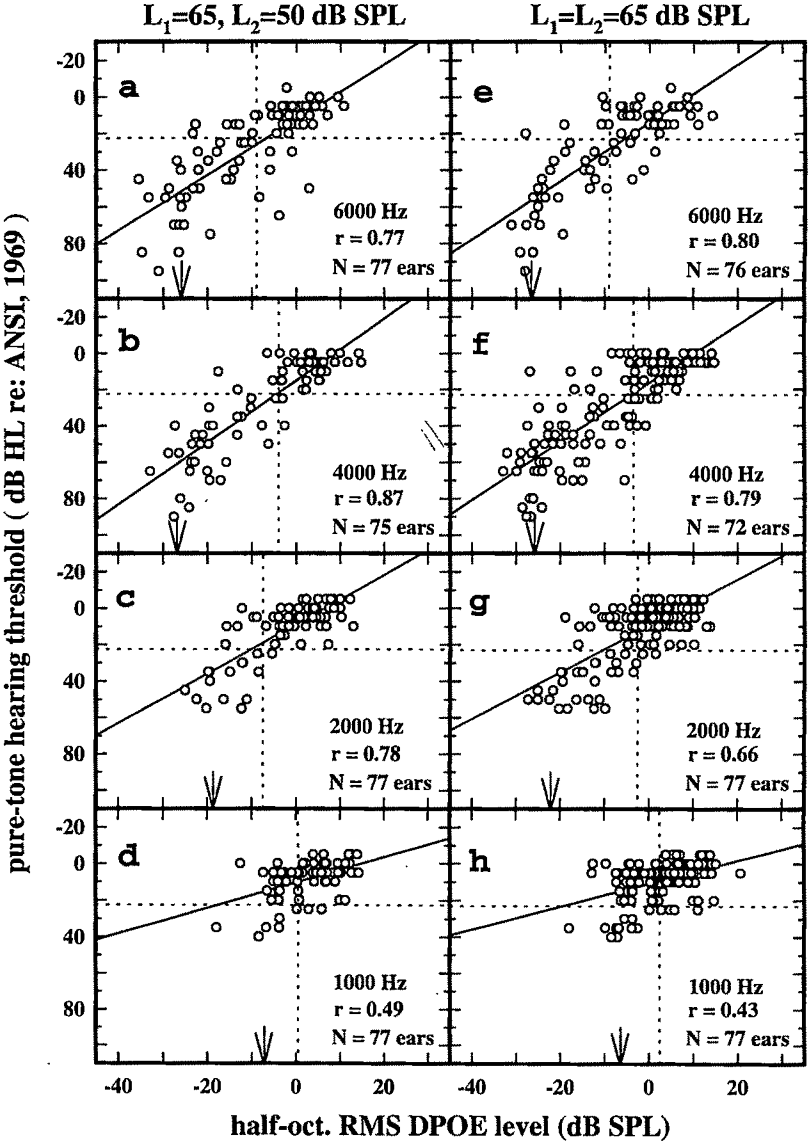

Fig 2. Relationship between pure tone hearing threshold and half-octave RMS DPOE level for group of human adult ears. For left column, L1 = 65 and L2 = 50 dB SPL, and right column, L1 = L2 = 65 dB SPL, pure tone audiometric frequency corresponded to f2 and $(f1 \times f2)^{0.5}$, respectively. Dotted horizontal line at 23 dB hearing level (HL) represents boundary separating normal versus impaired hearing thresholds, and vertical dotted line represents criterion DPOE level for DPOE test. Vertical arrow in lower left part of each panel represents average noise floor for frequency of measurement. Straight line running through data points is linear regression line. Number of tested ears (N) and correlation coefficient (r) are shown in panels.

included in this analysis for the following reasons. Data below 1,000 Hz had low signal-to-noise ratios, and half-octave RMS DPOEs for 8,000 Hz would require raw data at 0.25 octave above 8,000 Hz, which were not measured. Additional discussions about the 8,000-Hz condition are provided in the Discussion.

RESULTS

Correlation Analysis. A Pearson product-moment correlation coefficient[14] was applied to the relationship between the DPOE level and pure tone hearing threshold for a group of 77 ears with normal hearing or sensorineural hearing loss. The results are shown as scatter plots in Fig 2. The left and right columns represent the 65/50 and 65/65 stimuli. The four rows represent four different frequencies. The solid straight line running through the data points is a linear regression line. The correlation coefficient, r, ranged from .49 to .87 for 65/50 and .43 to .80 for 65/65. The positive regression line reflects a tendency for an ear with a better pure tone hearing threshold to exhibit a higher DPOE level. All of the correlations were statistically significant ($p < .01$). However, in terms of the percentage of predictable variance, the r^2 values were greater than .5 (ie, $r > .71$) only for some of the conditions, ie, 65/50 at three frequencies, except for 1,000 Hz, and 65/65 at 6,000 and 4,000 Hz. One may consider that an r value higher than .71 is statistically meaningful, since the independent variable can predict 50% or more of the variance of the dependent variable in such a case. In this regard, we consider that the correlation between DPOE level and pure tone hearing threshold is strong for the above conditions.

Performance of DPOE Test. In each panel of Fig 2, a criterion pure tone hearing threshold of 23 dB was used to separate normal from impaired hearing, as

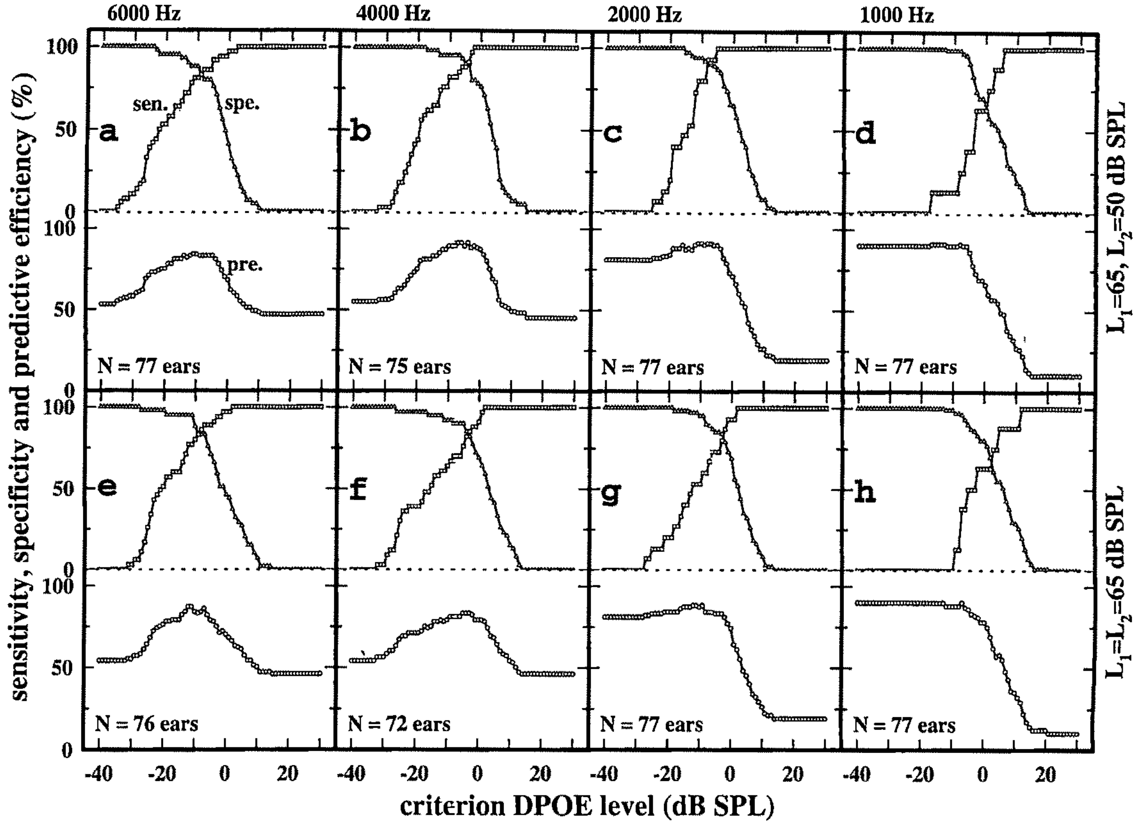

Fig 3. Sensitivity, specificity, and predictive efficiency versus criterion DPOE level for data obtained with L1 = 65, L2 = 50 dB, shown in upper half, and L1 = L2 = 65 dB in lower half. Results were derived from data shown in Fig 1.

indicated by a horizontal dotted line. This criterion is consistent with the definitions of normal hearing and hearing loss stated in Material and Methods. The present DPOE test of hearing loss corresponded to designating an ear to be of normal or impaired hearing, depending on whether the DPOE level was greater or less than a criterion DPOE level. A vertical dotted line in each panel of Fig 2 represents such a criterion DPOE level. With the two dotted lines, the panel is divided into four quadrants corresponding to four possible outcomes of the DPOE test as compared with the "gold standard" of pure tone hearing threshold, ie, true positive (lower left), true negative (upper right), false positive (upper left), and false negative (lower right).

The sensitivity, specificity, and predictive efficiency were computed from the number of data points in the four quadrants. The sensitivity, also called the hit rate, is defined to be the probability that a test correctly identifies the presence of a disorder such as a hearing loss.[14] Accordingly, the sensitivity was estimated to be [true positives/(true positives + false negatives)]. The specificity, also called the correct rejection, is defined to be the probability that a test correctly detects the absence of a disorder. Thus, the specificity was estimated to be [true negatives/(true negatives + false positives)]. The predictive efficiency is defined to be the overall probability for the outcome of a test to be correct. Thus, it was estimated to be [(true positives + true negatives)/all cases]. These measures of performance for the present DPOE tests are shown in the Table for the two stimuli at four frequencies. At the three highest frequencies, 6,000, 4,000, and 2,000 Hz, the values of sensitivity, specificity, and predictive efficiency of the DPOE tests were relatively high, 82% to 93%, for both of the two stimuli, while they were below 70% at 1,000 Hz. Comparing the two stimuli, the 65/50 stimulus yielded better performance at 4,000 and 2,000 Hz than the 65/65 stimulus in all of the three performance measures, while they were similar at 6,000 Hz and 1,000 Hz.

The values of sensitivity, specificity, and predictive efficiency were affected by the choice of a test criterion. In general, the sensitivity and specificity change inversely with the change of a test criterion. The effects of varying the criterion DPOE level on the three measures of test performance are shown in Fig 3. Here, the four columns represent the four frequencies, while the upper and lower halves represent the 65/50 and 65/65 stimuli. In the top and third rows, it can be seen that an increase of the criterion DPOE level led to an increase of the sensitivity and a

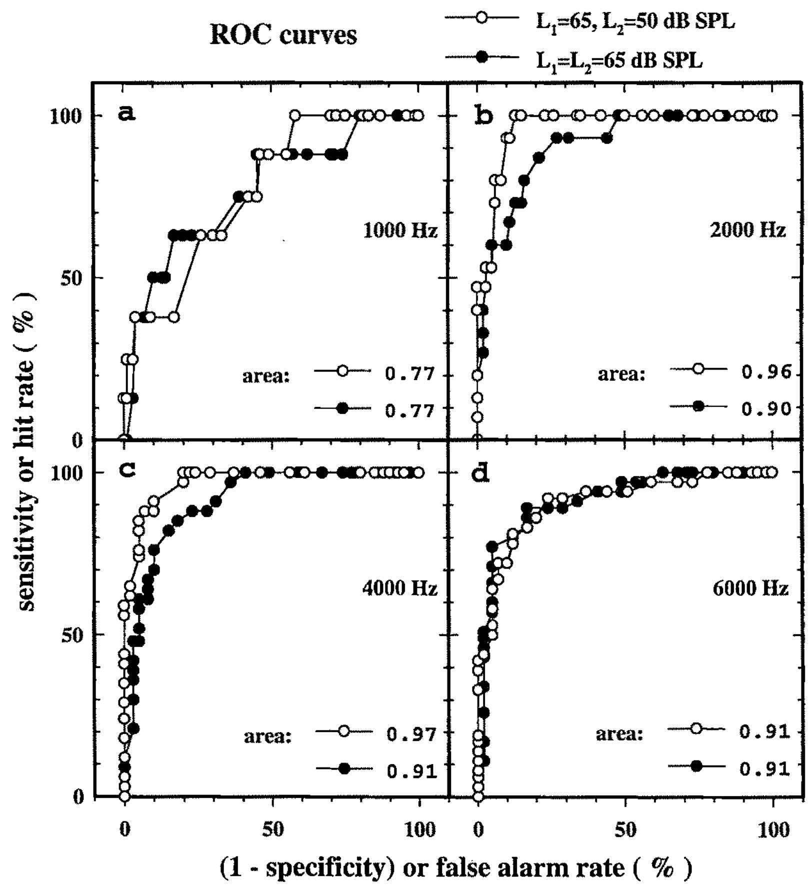

Fig 4. Receiver operating characteristic (ROC) curves corresponding to sensitivity, or hit rate, versus (1 − specificity), or false alarm rate of DPOE test of sensorineural hearing loss. Open and filled data points represent 65/50 and 65/65 stimuli, respectively. Area under ROC curve is indicated in each panel. These ROC curves were generated by replotting data shown in Fig 2.

decrease of the specificity. The value of the criterion DPOE level at the intersection of the sensitivity and specificity curves in Fig 3 was used in Fig 2 as the vertical line. These criterion DPOE levels are included in the Table. In the second and fourth rows of Fig 3, the predictive efficiency is shown initially to increase with increasing criterion DPOE level, generally reaching a peak and then declining. The predictive efficiency values in the Table were chosen at the criterion DPOE level close to the intersection of the sensitivity and specificity curves. The maximum of the predictive efficiency at the two lowest frequencies did not coincide with the intersection of the sensitivity and specificity curves, because hearing-impaired ears were fewer than normal ears in those cases. The unusual shape of the curves at the lowest frequencies has been discussed by Kim et al.[16]

Receiver operating characteristic is a graphical display of the sensitivity, or hit rate, of a test on the

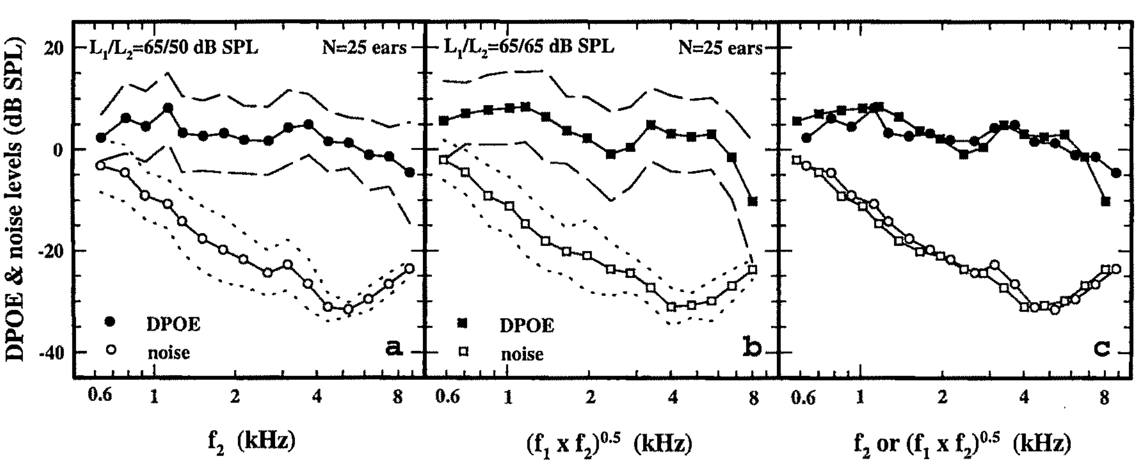

Fig 5. 2f1 − f2 DPOE level evoked by 65/50 (panel a) or 65/65 (panel b) stimulus versus frequency for 25 ears with normal hearing in subjects 18 to 35 years of age. Figure shows mean DPOE level ± 1 SD. Lower curves represent mean background noise level in tested ears ± 1 SD. Panel c is comparison of two mean values.

VARIOUS MEASURES EMPLOYED TO ASSESS PERFORMANCE OF DPOE TEST OF SENSORINEURAL HEARING LOSS AT FOUR FREQUENCIES FOR TWO SETS OF PRIMARY TONES

	65/50 dB SPL Stimulus (Hz)				*65/65 dB SPL Stimulus (Hz)*			
	6,000	*4,000*	*2,000*	*1,000*	*6,000*	*4,000*	*2,000*	*1,000*
Correlation coefficient	.77	.87	.78	.49	.80	.79	.66	.43
DPOE criterion (dB SPL)*	−9	−4	−8	0	−9	−4	−3	2
Total ears	77	75	77	77	76	72	77	77
True positive (ears)	30	31	14	5	29	27	12	5
False negative (ears)	6	3	1	3	6	6	3	3
True negative (ears)	34	37	56	46	34	33	52	48
False positive (ears)	7	4	6	23	7	6	10	21
Sensitivity (%)	83	91	93	63	83	82	80	63
Specificity (%)	83	90	90	67	83	85	84	70
Predictive efficiency (%)	83	91	91	66	83	83	83	69
ROC area	.91	.97	.96	.77	.91	.91	.90	.77

DPOE — distortion product otoacoustic emission, SPL — sound pressure level, ROC — receiver operating characteristic.

*DPOE criterion was chosen to yield nearly same values of sensitivity and specificity.

ordinate versus (1 – specificity), or false alarm rate on the abscissa. A useful quantitative index of an ROC is the area under the ROC curve. It represents a probability that a randomly chosen pair of diseased and normal subjects will be correctly ranked by a test regarding the subjects' disease.[24] From the data in Figs 2 and 3, we constructed the ROC curves shown in Fig 4. The greater the area under the curve, the better the test performance. The areas under the ROC curves were 0.90 or larger at the three highest frequencies for both of the two stimuli. The areas were smaller at 1,000 Hz (0.77). Comparing the two stimuli, the 65/50 stimulus yielded larger ROC areas (.96 and .97) at 4,000 and 6,000 Hz than the 65/65 stimulus (.90 and .91). A Wilcoxon test was employed to evaluate whether the differences between the ROC areas obtained with the two stimuli at these two frequencies were statistically significant. The results revealed that the differences were significant, with $p < .001$.

Results in Normal-Hearing Ears. Data from 25 ears with normal hearing in subjects 18 to 35 years old were used to provide descriptions of normative DPOE baseline for the 65/50 and 65/65 stimuli. Mean DPOE and background noise level with ±1 SD are shown as a function of frequency in Fig 5. The mean DPOE level ranged from 8 dB near 900 Hz to −5 dB near 8,000 Hz for the 65/50 stimulus, and from 8 dB near 1,000 Hz to −10 dB near 8,000 Hz for the 65/65 stimulus. With respect to the noise floor, it was lower in higher frequencies, becoming progressively higher for lower frequencies. The signal-to-noise ratio was mostly high in the middle to high frequencies (20 to 35 dB), while it was low at frequencies below about 1,000 Hz.

DISCUSSION

The main finding of the present study is that a DPOE test of human sensorineural hearing loss with $L_1 = 65$ and $L_2 = 50$ dB SPL yielded a better performance than that with $L_1 = L_2 = 65$ dB SPL at 2,000 and 4,000 Hz. This was the case in terms of all measures of test performance evaluated: area under the ROC curve (.96 to .97 versus .90 to .91), sensitivity/specificity (90% to 93% versus 80% to 85%), and correlation coefficient (.78 to .87 versus .66 to .79) (see Table and Figs 3 and 4). The two stimuli yielded similar test performances at 1,000 and 6,000 Hz. Thus, the 65/50 stimulus provided a better overall performance than the 65/65 stimulus. To our knowledge, the present study is the first to compare directly the performances of DPOE tests of human sensorineural hearing loss with stimuli having $L_1 > L_2$ and $L_1 = L_2$. From the present study, we recommend the use of stimuli with $L_1 > L_2$ by about 15 dB in a DPOE test of human sensorineural hearing loss. The present finding is consistent with the prediction from the animal studies of Mills et al.[12] Whitehead et al[25] recently reported a finding similar to that of Mills et al. The present finding is also consistent with a recent human study that there was a greater reduction of DPOE level in ears with sensorineural hearing loss when $L_1 > L_2$.[13]

Decision theory offers a useful approach to evaluating the performance of a new test against a standard test. Among several measures of test performance, ie, sensitivity, specificity, predictive efficiency, and area under the ROC curve, the last measure, in particular, offers the advantage of characterizing the overall performance of a test independent of the setting of a test criterion. The present study confirmed findings of earlier, closely related studies. For example, Gorga et al,[15] using $L_1 = 65$ and $L_2 = 50$ dB SPL, and Kim et al,[16] using $L_1 = L_2 = 65$ dB SPL, observed that DPOE tests provided relatively high measures of test performance. The present results of sensitivity and specificity for frequencies 2,000 to 6,000 Hz and combining the 65/50 and 65/65 results, 82% to 93%, were comparable to those of Gorga et al (82% to 92%) and of Kim et al (82% to 89%). In terms of ROC areas, the three sets of results are .90 to .97 (present), .86 to .95 (Gorga et al), and .91 to .94 (Kim et al). Since the Gorga et al and Kim et al studies each used a different one of the two stimuli being considered here, the two sets of results may conceivably help compare the test performances using the two different stimuli. However, the fact that the two sets of results were obtained in two separate studies would make a detailed comparison difficult. Thus, we suggest that the present results obtained from a common group of subjects is more useful in evaluating the question.

Correlation coefficients, r's, between pure tone hearing threshold and DPOE level found in this study were comparable to those observed previously. The present values of r, .77 to .87 for the 65/50 stimulus and .66 to .80 for the 65/65 at 2,000 to 6,000 Hz, are comparable to those in the literature: .67 to .89 at 4,000 to 8,000 Hz,[26] .85 and .71 at 4,000 and 8,000 Hz,[15] and .76 to .83 at 2,000 to 6,000 Hz.[16] In all of these studies, lower r's of .43 to .55 were observed at 1,000 Hz. Other investigations[26,27] found that the DPOE *detection threshold* (as opposed to the DPOE *level*) also showed comparably strong correlations with pure tone hearing threshold.

All adult ears with normal hearing in our sample showed detectable DPOEs for the entire measured frequency range. This result is consistent with previous studies.[6,7,19,28] The general appearance of the mean DPOE level-versus-frequency function in the present study, depicted in Fig 5, is comparable to those reported previously.[6,18,29,30] One difference we noted is that for the stimulus pair of 65/50, the results of Gorga et al[15] in 80 normal ears showed a higher noise floor, especially at 1,000 Hz and below (eg, 14 dB at 537 Hz), than the present report (eg, –3 dB at 584 Hz). The reason for this difference is not clear.

This study agreed with others that DPOE test performance was worse at frequencies of 1,000 Hz or lower. This is attributed to a nearly monotonically increasing noise level with decreasing frequency and a resulting degradation in the DPOE signal-to-noise ratio in these low frequencies (Fig 5). In addition, a small number of hearing-impaired ears at low frequencies in the present sample made the present results less reliable at 1,000 Hz regarding test performance. At 8,000 Hz, we encountered a greater variability than at 6,000 or 4,000 Hz. Errors arising from standing-wave nodes in estimating the levels of the primary tones and DPOEs expected at this high-frequency region[31,32] may underlie a greater variability. Therefore, future efforts may be directed to both the low end of the frequency spectrum, seeking an improvement in DPOE signal-to-noise ratio, and the high end of the spectrum, seeking an improved method of specifying the levels of the stimulus and DPOEs.[32]

ACKNOWLEDGMENTS — The invaluable and patient participation of our subjects in the measurement is gratefully acknowledged. We thank Dr K. Parham, Dr J. Smurzynski, Dr H. Zhao, S. Ghoshal, and J. Paparello for their assistance in data analysis, and Dr J. L. Clive, Biostatistician, for his help in applying the statistical tests.

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INTERAMERICAN ASSOCIATION OF PEDIATRIC OTORHINOLARYNGOLOGY

The biannual meeting of the Interamerican Association of Pediatric Otorhinolaryngology will be held in Sao Paulo, Brazil, September 21-24, 1997. For information, contact Office of the Secretary, Tania Sih, MD, PhD, Rua Itapeva, 366, Suite 102, 01332-000, Sao Paulo SP, Brazil; telephone 55 11 283-4645 or 283-3396; fax 55 11 826-9652 or 542-6037.

IMAGING CASE STUDY OF THE MONTH

SIGMOID SINUS THROMBOSIS AND IMAGING TECHNIQUES

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INTRODUCTION

Septic thrombosis of the sigmoid sinus has a small incidence among intracranial complications of otitis media. It is the complication most frequently represented after meningitis, epidural abscess, and brain abscess.

The use of antibiotics has not only dramatically reduced its frequency, but has influenced in many cases the clinical presentation, increasing the difficulty of its diagnosis. Because of the potential lethal evolution of this disorder, it is mandatory that an early diagnosis be made in order to establish adequate treatment for resolution. With this in view, imaging techniques, with particular emphasis on the use of magnetic resonance imaging (MRI), can provide valuable information on the nature of this lesion and its extent.

In this report we present a case of sigmoid sinus thrombosis, analyzing the contemporary diagnosis and management of this uncommon pathologic condition.

CASE REPORT

A 71-year-old man with a 22-year history of chronic otitis media in his right ear was admitted to our department complaining of an intermittent purulent right otorrhea of 3 days' duration.

The patient was febrile to 37.8°C. No neurologic deficit was detected. He had severe diabetes mellitus with a blood sugar level to 320 mg/dL. Otoscopy showed purulent otorrhea emanating from a right tympanic membrane perforation. A contrast-enhanced computed tomography (CT) scan revealed a massive opacification of the right mastoid with extensive

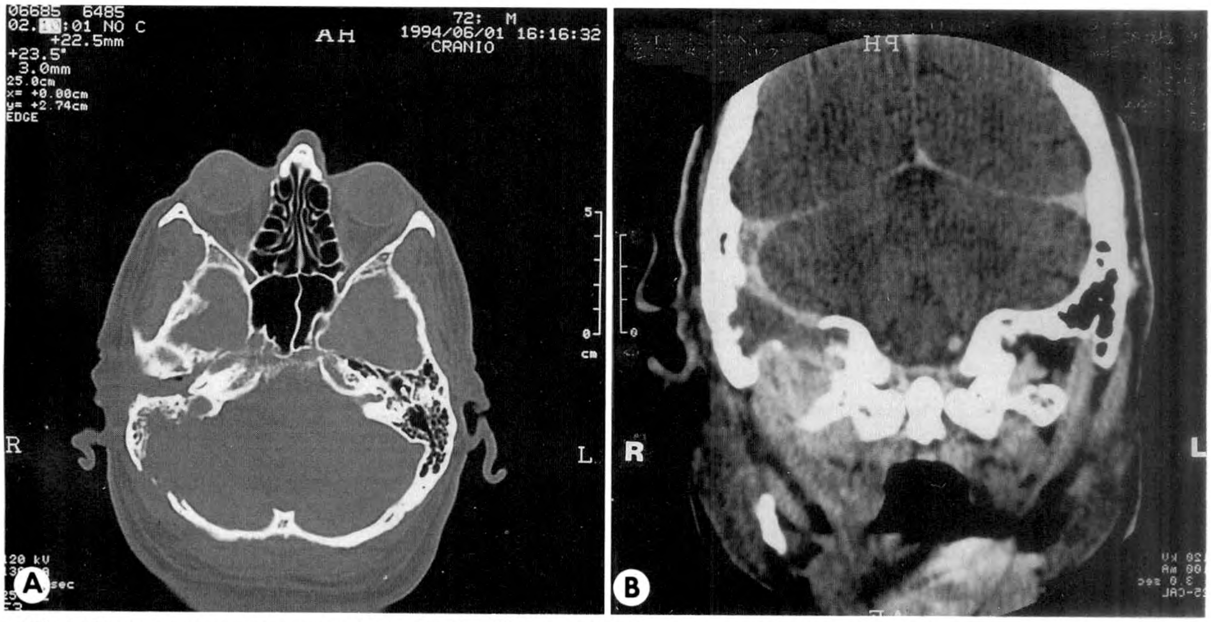

Fig 1. Contrast-enhanced computed tomography. A) Axial view shows extent of erosion of mastoid. B) Coronal view shows wide erosion of mastoid, with inflammatory tissue occupying entire mastoid and jugular foramen, and dural inflammatory reaction.

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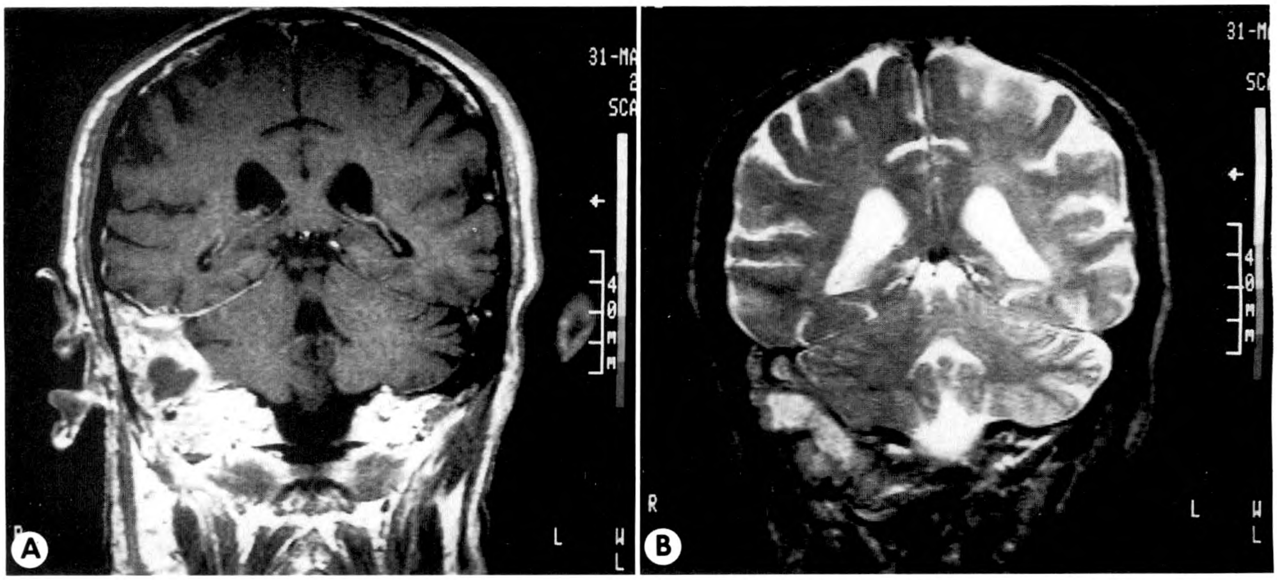

Fig 2. Magnetic resonance imaging. A) T1-weighted coronal view shows thrombosis of lateral sinus, distal segment of transverse sinus, and proximal portion of jugular vein. B) T2-weighted coronal view shows inflammatory tissue with extensive liquefaction necrosis involving entire mastoid.

bone erosion. There were no direct signs of sigmoid sinus thrombosis (Fig 1). Magnetic resonance imaging was performed that demonstrated a low-intensity signal from the sigmoid sinus, consistent with thrombosis. This was associated with concomitant thrombosis of the distal segment of the transverse sinus and the proximal tract of the jugular vein (Fig 2). The lesion caused slight compression of the ipsilateral cerebellar hemisphere.

The patient underwent a complete right mastoidectomy. At operation the mastoid was thoroughly filled by granulation tissue and purulent debris. We also found a marked erosion of the bony plate overlying the sigmoid sinus and posterior fossa dura. The sinus wall, covered with granulation tissue, was incised and the intraluminal thrombus was evacuated. The patient had an excellent postoperative recovery and after a 7-day hospitalization was discharged home. At 1-year follow-up he was symptom-free with no neurologic deficits.

DISCUSSION

Although the introduction of antibiotics in the treatment of septic thrombophlebitis of the dural venous sinuses has reduced the incidence of sigmoid sinus thrombosis and has changed its clinical evolution, this disorder is still a potentially fatal complication of ear infection.

In a review of the literature, its mortality is indicated in approximately 10% of cases. It should also be remembered that in 10% of the patients the recovery is complicated by residual neurologic deficits.[1-6]

The widespread use of antibiotics has altered the clinical presentation of sigmoid sinus thrombosis, with an increase in nonspecific symptoms (headache, low-grade fever, general malaise, increased otorrhea in chronic otitis media).[7-9] This subtle clinical course requires more attention on the part of the otolaryngologist, in order to expedite diagnosis and treatment and hence avoid lethal evolution or crippling sequelae. Our case is a paradigmatic example of this potentially misleading condition with its minimal and vague symptoms and signs.

Advances in imaging techniques have facilitated the early diagnosis of sigmoid sinus thrombosis. On CT scanning, an empty triangle appearance at the level of the sinus surrounded by contrast-enhanced dura, the so-called "delta sign," is described as a typical sign of the thrombosis.[10,11] However, this sign is not always detectable, and not all thromboses are documented by CT.

McMurdo et al,[12] Harris et al,[13] McArdle et al,[14] and Kelly et al,[15] reviewing the English-language literature, noted that in no cases was thrombosis within the sigmoid sinus visualized by CT. Our case confirms such observations, showing no direct CT evidence of the sigmoid sinus thrombosis.

Magnetic resonance imaging is more sensitive in detection of the disorder. All of the published cases were preoperatively identified by MRI.[15,16] It is the imaging method of choice for its superior ability to evaluate blood flow, being able to demonstrate sinus obstruction and eventual reversal of flow.[11] This aspect, combined with its higher resolution in detailing nerve tissue, allows an earlier and more precise diagnosis of this lesion, delineating its extent and involvement of the surrounding structures. Gadolinium enhancement of MRI scanning depicts the specific pattern of the thrombosis. It is detectable (see Case Report) as a soft tissue signal associated with a vascular and bright appearance of the dural sinus walls.

The rationale of treatment demands surgical removal of the septic mastoid focus through opening of the sinus and intraluminal evacuation of the thrombus. This aggressive management improves the prognosis of a septic sigmoid sinus, with better chances of a quick and uncomplicated recovery.[11]

In conclusion, the present case illustrates that clinical suspicion of this complication of chronic otitis media, in combination with appropriate imaging techniques and prompt surgical intervention, allows favorable resolution of this potentially lethal disorder, even when the general medical status is not ideal, as in our patient, who had the concomitant presence of severe diabetes.

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THIRTY-SECOND POSTGRADUATE COURSE IN EAR SURGERY

The Thirty-Second Postgraduate Course in Ear Surgery will be held in Nijmegen, the Netherlands, from March 31 to April 5, 1997. For information, contact Prof P. van den Broek, University Hospital Nijmegen, Department of Otorhinolaryngology, PO Box 9101, 6500 HB Nijmegen, the Netherlands; fax 024-3540251.

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IMAGING CASE STUDY OF THE MONTH

AEROCELE: AN UNUSUAL COMPLICATION OF TRACHEOCUTANEOUS FISTULA REPAIR

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CASE REPORT

A 3-year-old male, 27-week ex–premature infant had a history of bronchopulmonary dysplasia, seizure disorder, and developmental delay. He required tracheostomy and was on ventilatory support for approximately 10 months. After decannulation, he continued to have a persistent tracheocutaneous fistula necessitating surgical closure. His repair consisted of excision of the skin and underlying scar tissue down to the anterior tracheal wall. The trachea was not entered but was oversewn. The remaining overlying tissue was closed over the trachea. A Penrose drain was placed, and the skin was reapproximated. The drain was removed after 24 hours, and the child was discharged.

Two weeks later, the patient presented with a 1-week history of an increasing midline neck mass directly over the incision. The patient's mother noted that when the child coughed or cried, the mass appeared to enlarge. Over the preceding 2 days, the parents had noted erythema and increasing tenseness of the overlying skin. The parents denied any respiratory distress. The patient had not been febrile. There was no drainage from the neck.

On physical examination, the patient was afebrile with a normal respiratory rate for age. Examination of his neck revealed a 2.0 × 1.5-cm partially compressible mass in the midline of the old tracheostomy incision. The overlying skin was erythematous and tense (Fig 1). There was no evidence of fluctuance or drainage. There was no evidence of crepitus in the surrounding tissues. The rest of the findings on head and neck examination were unremarkable. Computed tomography (CT) with intravenous contrast was performed to rule out the possibility of an abscess. The CT revealed a pocket of air in the subcutaneous tissue overlying the anterior tracheal wall (Fig 2).

The patient was admitted and given intravenous antibiotics. He underwent excision of the lesion and placement of a drain in the operating room the next day. His drain was removed after 24 hours, and he

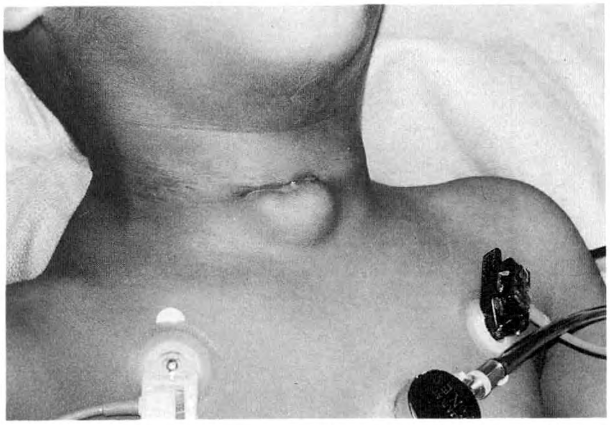

Fig 1. Patient in supine position revealing protruding midline neck mass.

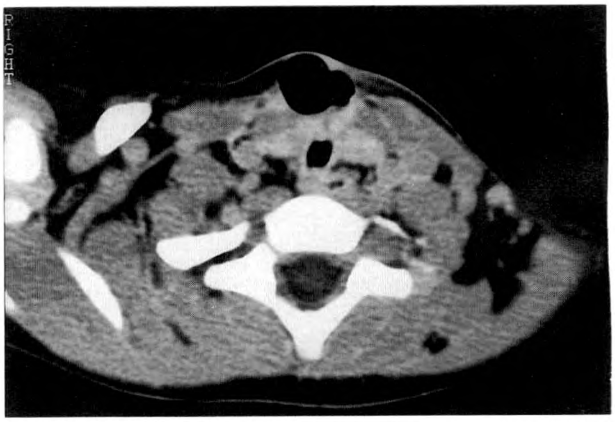

Fig 2. Coronal computed tomogram showing evidence of collection of air, several centimeters in diameter, anterior to tracheal lumen.

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was discharged. Two weeks later, the aerocele recurred. Subsequent reexploration included reidentification of the fistulous scar tract, placement of a purse-string suture, oversewing of the anterior tracheal defect, and a double layer closure of the strap muscles. The patient did well, without evidence of recurrent aerocele or tracheocutaneous fistula.

DISCUSSION

Tracheocutaneous fistula is a complication of prolonged tracheostomy and has been shown to be directly related to length of cannulation.[1] Significant complications may occur secondary to tracheocutaneous fistula closure.[2,3] Subcutaneous emphysema, pneumothorax, pneumomediastinum, pneumopericardium, and airway compression are all emergencies.[4,5] Less severe complications may also occur. Aerocele is one such unusual complication. This case is the first reported of CT-documented aerocele after tracheocutaneous fistula repair in the otolaryngology literature. Other reports of aerocele have been described after anterior cricoid split and laryngotracheoplasty procedures.[6,7]

The mechanisms of aerocele formation are similar in all instances. A small defect in the anterior tracheal wall acts as a ball valve, allowing air to escape into the overlying soft tissues. This accumulation of air usually progresses anteriorly, creating tense overlying skin. If increased pressure persists, this may extend into the incision site, re-creating a tracheocutaneous fistula.

Treatment is relatively straightforward, involving excision, closure of the defect, and placement of a drain. Normal saline and positive pressure ventilation can be used to localize the leak, but this is not always successful. Generally, the drain can be removed within 24 to 48 hours, after a seal has formed over the anterior tracheal wall. The role of repeated aspiration with or without a compression dressing has not been evaluated in this unusual condition.

Aerocele is an important, however rare, treatable complication of tracheocutaneous fistula repair that is recognizable both clinically and by radiologic evaluation such as by CT scan.

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NEUROMONITORING IN OTOLARYNGOLOGY AND SKULL BASE SURGERY

Neuromonitoring in Otolaryngology and Skull Base Surgery will be held in London, England, February 21, 1997. For information, contact Dianne Rooksby, Centre for Continuing Professional Development, University of Nottingham, University Park, Nottingham, NG7 2RD, United Kingdom; telephone 44 115 951 3763; fax 44 115 951 3722; Email dianne.rooksby@nottingham.ac.uk.

Ann Otol Rhinol Laryngol 105:1996

PATHOLOGY CONSULTATION

THYROID GLAND ECTOPIAS

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Failures of descent of the medial anlage of the thyroid and incomplete obliteration of its vertical tract lead to midline or near-midline ectopias such as lingual thyroid and thyroglossal cysts. Each poses special diagnostic and therapeutic considerations. "Ectopias" of the thyroid gland lateral to the carotid artery and jugular vein, however, cannot be readily explained by current embryological information. In these instances, and especially for intranodal thyroid tissue, a metastasis from an occult thyroid primary is the mandatory first consideration.

INTRODUCTION

Ectopias of the thyroid gland in the head and neck have been the subjects of an aggregation of medical literature. In the following, after an admittedly brief embryological introduction,[1-4] we present a synopsis of 1) lingual thyroid, 2) thyroglossal cysts, and 3) lateral aberrant thyroid tissue (see Figure).

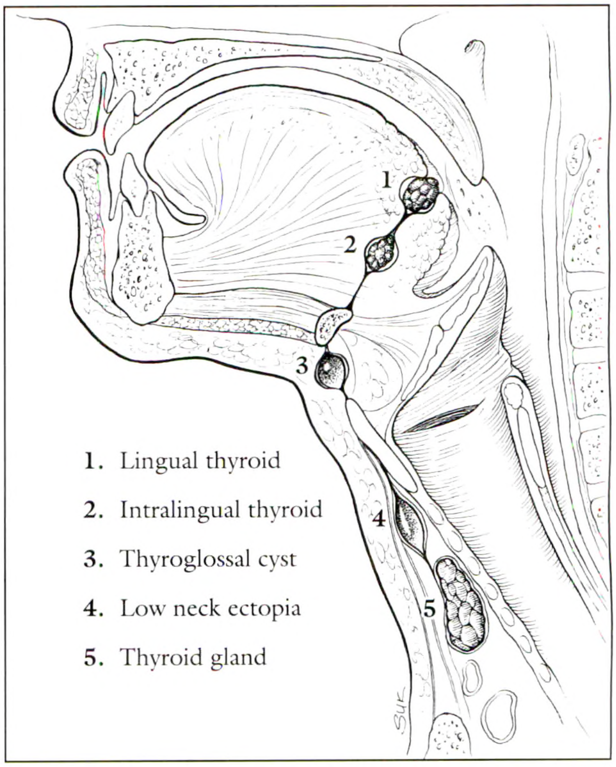
The thyroid gland develops as the first pharyngeal derivative as an endodermal diverticulum in the midline of the ventral pharynx between the first and second pharyngeal pouches. Lobulation of the diverticulum occurs and it is connected to the floor of the pharynx by the thyroglossal duct, where the pharyngeal contribution to the tongue is sited. The anlage follows the descent of the heart and great vessels and grows caudally into the loose prepharyngeal connective tissue and passes anterior to the developing hyoid bone. By the 7th embryonic week, a now-concentric thyroid comes to lie anterior to the developing trachea. By then, the thyroglossal duct has begun to atrophy, with the original pharyngeal connection of the duct identified at the apex of the sulcus terminalis on the dorsum of the tongue — the foramen cecum.

The median anlage yields most of the thyroid parenchyma. Two lateral anlagen, products of the fourth pharyngeal pouches, contribute the parafollicular C-cells and perhaps 1% to 30% of the thyroid weight.

LINGUAL THYROID

Lingual thyroid is a developmental anomaly characterized by an aggregate of thyroid tissue in the midline of the base of the tongue, between the circumvallate papillae and the epiglottis. Its size varies from a few millimeters to several centimeters, and symptoms and signs are nearly always related to the size. Symptomatic lingual thyroid tissue is, however, quite unusual, with approximately 400 reported patients.[5] This is to be compared with 1) an estimated prevalence of *grossly* visible lesions of 1:10,000[4] and 2) a postmortem study yield of 10% (equally distributed between males and females).[6] Sublingual or partially descended thyroid tissue is far less common.

A 1972 study by Baughman[4] remains the unrivaled



Schematic illustration of location of midline ectopias of thyroid gland.

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document on the clinical and histopathologic aspects of the lingual thyroid and lingual thyroglossal duct remnants. From his report and others that have followed, a clinicopathologic synopsis can be presented.

The majority of lingual thyroid tissues are asymptomatic and are usually found on routine oral examination. As indicated earlier, 1 in 10 subjects may have microscopic lingual remnants of thyroid tissue. Symptomatic lesions may appear at any time from birth to old age. For those present at birth, it is likely that all or nearly all of the thyroid tissue has remained in the tongue.[7] The tongue is the most usual site for a total ectopy of thyroid. The majority of symptomatic lingual thyroids arise during puberty, pregnancy, or menses and hence are more common in women in a ratio of 7:1 over men.[7,8] The mean age when clinical manifestations occur is 40 years (with two statistical peaks at 12 and 50 years of age).[6,7] Signs and symptoms are largely related to the size of the lingual thyroid and consist of dysphagia, dysphonia, dyspnea, and occasionally hemorrhage. Large masses can be life-threatening because of obstruction. Ten percent of young patients manifest juvenile myxedema or cretinism.[3] Approximately 70% of patients will have various degrees of hypothyroidism, and between two thirds and three quarters of patients with symptomatic lingual thyroids have no other functional thyroid tissues.[4,8] It has been claimed that there is a high incidence of thyroid disease in family members of patients with lingual thyroid.[9]

The lingual thyroid may be circumscribed, but in nearly all instances, thyroid tissue is found interdigitating with skeletal muscle and the minor salivary gland tissues in the tongue's base. In a minority of the reported examples, the thyroid tissue extends through the entire thickness of the base of the tongue, with or without thyroglossal tract remnants.[4] Heir to any disease or pathologic change seen in the normally placed thyroid gland, the lingual thyroid usually shows embryonic thyroid tissue, mature thyroid tissue, or a combination of these.

Investigation of a symptomatic lingual thyroid begins with a thorough examination of the oropharynx and neck for clinical evidence of a normal thyroid position. Radionuclide technetium 99m and iodine 131 or 123 thyroid scans are nearly mandatory.[7] Computed tomography can be helpful in delineating the extent of the lesion.[7]

Carcinoma in Lingual Thyroid. Twenty-three cases of thyroid carcinomas arising in a lingual thyroid had been reported by 1992; none were papillary in type.[10] The majority were follicular carcinomas. At the time of reporting, 4 patients had distant metastasis and 5 had suffered recurrences.[10] Most of the carcinomas occur in the third decade of life. The gender ratio is nearly equal. It should be noted that the rate of malignant transformation in ectopic thyroid tissue is no greater than in the normally placed thyroid gland.

THYROGLOSSAL DUCT REMNANTS

The thyroglossal tract usually undergoes atrophy and disappears between the 5th and 10th embryonic weeks.[2,11] The caudal attachment of the tract may persist as a pyramidal lobe of the thyroid gland. Thyroglossal cysts arise as cystic expansion of remnants of the tract. They may, on occasion, be multiple. The finding of a thyroglossal cyst implies a thyroid gland in a more distal location, since the gland is in the van of the descent of the anlage.

There is an equal sex distribution in subjects with thyroglossal cysts. Allard,[2] after reviewing a series of 1,316 cases from the literature, indicated that 31.5% of patients were under the age of 10, 20.4% were in their second decade, 13.5% were in their third decade, and 34.6% were older than 30 years. Over half the patients were in their first or second decade.

The vertical location of a thyroglossal cyst can be placed as follows: 2.1% are intralingual, 24.1% are suprahyoid (including submental), 60.9% are between the hyoid bone and the thyroid cartilage (thyrohyoid), and 12.9% are substernal.[2] Between 60% and 80% present below the hyoid bone.[2] Cysts below the level of the hyoid bone are almost always embedded in strap muscles.[2] Only a thyroglossal cyst is situated between the strap muscles and the lateral aspect of the thyroid cartilage.[12] In contrast, a branchial cyst lies just anterior to the sternocleidomastoid muscle and lateral to the carotid artery.

The primary presenting clinical feature is an asymptomatic, slowly enlarging midline cystic mass. Eccentric or lateral presentation is most often due to infection or prior manipulation of the cyst.[13] An unadulterated thyroglossal remnant is rarely associated with a sinus or fistula. Most sinuses are secondary to incomplete surgical removal or infection.[2,13]

The histopathology of a thyroglossal cyst centers on the lining of the cyst and also its wall. Various types of linings can be found, ranging from a denuded surface to a pseudostratified ciliated columnar, cuboidal, or flattened or squamous epithelium.

Thyroglossal cysts above the hyoid are more often lined by squamous epithelium, and those below the hyoid, more often by a columnar epithelium. The epithelial contents of the wall are principally salivary (mucous or seromucous) or thyroid tissue. Frequencies of 1.5% to 65% have been reported for the former.[14] The degree of diligence in searching may

NECROPSY DATA, ACCORDING TO GEOGRAPHIC LOCATION, ON OCCULT PAPILLARY CARCINOMA OF THYROID GLANDS

Country	*% Occult Carcinomas*
Finland	35.6
Japan	28.4
Spain	22.0
United States (Michigan)	13.0
Poland	9.0
Canada	6.0
Colombia	5.6

Modified from Martinez-Tello et al.[25] Percentages are based on at least 100 necropsies (range, 100 to 607).

account for this wide variation. We have been impressed with the frequency of salivary tissue found in the walls of thyroglossal duct cysts. The closer the cyst to the lingual area, the greater the percent. It is our impression that salivary tissue is as frequent as thyroid tissue. The flattened epithelium will often be colored in immunocytochemical studies with antibodies against thyroglobulin, and this lining, rather than the mural thyroid tissue, may be the source of neoplasms found in thyroglossal cysts.

Thyroglossal Carcinoma. Thyroglossal carcinoma occurs in less than 1% of thyroglossal cysts. Approximately 150 cases have been described in the literature since 1911.[15] There does not seem to be a gender predilection, and the average age of patients lies in the fourth decade, but there is a wide range (1 to 82 years). The majority of thyroglossal carcinomas are located in the thyrohyoid region. Papillary carcinoma is, by far, the leader of histologic subtypes and approaches 95% of all reported cases (includes "mixed" forms).[16] Follicular carcinoma is rare,[17] and anaplastic,[15] rarer still. In contrast to its low frequency in the thyroid gland proper, squamous cell carcinoma is the second most commonly reported subtype.[18] There have not been any medullary carcinomas reported as arising from thyroglossal tract remnants. This is expected, because of the ultimobranchial origin of the C-cells.

The papillary carcinomas may be confined to a cystic enclosure or invade strap muscles, an event we feel is more frequent than the reported 4%.[16] In a noteworthy difference from thyroid gland papillary carcinomas, the incidence of metastasis to cervical lymph nodes from a thyroglossal carcinoma is low and has been estimated at 8%.[16,17,19]

LATERAL THYROID ECTOPIA

Controversy over lateral aberrant (ectopic) thyroid tissue has been with us for over two centuries and has been flogged in the literature almost as often as the branchiogenic carcinoma story. Lateral must be defined as lateral to the carotid sheath and jugular vein.[20] With that restriction, any thyroid tissue within a lymph node, regardless of its "normal" appearance, is metastatic from a thyroid gland primary and is not a developmental anomaly.[20] Apparently aberrant thyroid tissue, unassociated with lymph nodes, can be seen in sequestered nodules, through implantation by prior surgery or trauma, or as a result of an intimate association of the *median anlage* during development with mesodermal structures in the neck, eg, muscle, fibroadipose tissue, carotid sheath.[20,21] Such nodules should *not* have papillae or psammoma bodies, appear histologically normal, or, in the case of sequestration, appear adenomatous or as bearing a thyroiditis.

The ultimobranchial (or lateral) contribution to the thyroid gland is known to derive from the fourth and fifth branchial pouches and to descend with parathyroid IV. In the normal human fetus (but not in lower vertebrates) the ultimobranchial component fuses with the thyroid gland. Abnormalities in the descent or failure of fusion of the ultimobranchial body have been posited as explanations for lateral aberrant thyroids, especially since Williams et al[22] showed the ultimobranchial body could contribute follicular cells as well as C-cells to the human thyroid gland. Clinical experience, however, has nearly completely negated this thesis.[23] Patients with undescended thyroid rests such as lingual thyroids do not show radioiodine uptake in the lateral paratracheal areas, and we are unaware of an extrathyroidal primary medullary carcinoma of the thyroid in the lateral neck.

A special diagnostic problem occurs as a result of histologic differentiation of papillary thyroid carcinoma in a metastatic site and also by cystic dilation of a metastasis. With the latter, only the rare papillary focus betrays the carcinoma in an otherwise flattened thyroid epithelial lining. In both instances, there may not be identifiable lymph nodal tissue.

Microscopically normal thyroid follicles can be found in lymph nodes not lateral to the carotid sheath, and here the assumption of a metastasis may be contested even though there are no good embryological or anatomic reasons for such inclusions. Meyer and Steinberg[24] offer histopathologic clues that they feel characterize benign inclusions: 1) a microscopic size, adjacent to or in the lymph node capsule, 2) uncrowded, nonpapillary follicles, 3) no enlargement of nucleoli in uncrowded, nonenlarged nuclei, 4) no associated stromal proliferation, and 5) no psammoma bodies.

The frequency of clinically latent or occult thyroid carcinomas found 1) coincidentally after surgery for a goiter, 2) as part of a surgical procedure for non-thyroid carcinoma of the head and neck, or 3) in necropsy studies (see Table[25]) is higher than the annual incidence of clinically apparent thyroid carcinomas.[25] Most are papillary in type, and as Chen[26] has noted, any papillary carcinoma of the thyroid gland, no matter how small (even less than 1 mm), has a significant metastatic potential. There does not appear to be a tendency of these small carcinomas to increase in size toward a clinically apparent tumor with age. These observations also speak more to the likelihood of a metastasis rather than an ectopia in the lateral neck. So does the presence of psammoma bodies.

The diagnostic significance of psammoma bodies for papillary carcinoma of the thyroid gland is well known.[27,28] Even bodies found in lymphatics of the thyroid adjacent to tumors or in the opposite lobe, as well as in some regional lymph nodes, represent calcified metastatic tumor emboli. Johannessen and Sobrinho-Simoes[28] indicate that the presence of psammoma bodies in serous tumors of the ovary or papillary thyroid carcinomas indicates a good prognosis.

TREATMENT

Treatment of a symptomatic lingual thyroid depends on its size, the severity of symptoms, the presence of thyroid tissue besides that in the tongue, and complications such as ulceration, hemorrhage, or malignancy. Most patients respond to thyroxine suppression alone. For those who do not, surgical removal or iodine ablation with or without autotransplantation should be considered.[7]

A history and physical examination pointed toward finding a mild hypothyroidism, routine thyroid function tests (pertinently, thyroid stimulating hormone), and a judicious use of a preoperative thyroid scan are the recommended evaluations for pediatric patients with a presumptive thyroglossal cyst.[29]

The Sistrunk procedure is the obligatory start for thyroglossal cysts and thyroglossal carcinomas.[11,13,17,29,30]

Early surgical removal to avoid subsequent infection is advocated for thyroglossal cysts.[26] The Sistrunk operation, in which the cyst, tract, central part of the hyoid bone, and a core of soft tissue from the tongue base are included, is the requisite procedure. Morbidity is minimal, postoperative complications are generally minor, and recurrences are minimized (less than 5%).[29]

Individualization of treatment beyond a Sistrunk procedure for thyroglossal carcinoma depends on the histologic type of the carcinoma and extent of invasion. Palpable abnormalities in the thyroid gland and clinical evidence of involved lymph nodes should lead to a consideration of total thyroidectomy and appropriate neck dissection with subsequent iodine 131 ablation.[31] Coincident carcinoma of the thyroid gland proper has ranged from 0% to 25%.[17] The likelihood of the midline thyroglossal carcinoma's being a metastasis is, however, low. After appropriate treatment, recurrences of a thyroglossal papillary carcinoma are uncommon and metastases are far less frequent than noted with papillary carcinoma of the thyroid gland. Death due to a papillary thyroglossal carcinoma is practically unknown.

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THE SEVENTEENTH EUROPEAN INSTRUCTIONAL COURSE ON TINNITUS AND ITS MANAGEMENT

The Seventeenth European Instructional Course on Tinnitus and Its Management will be held in Nottingham, England, on April 13-17, 1997. For information, contact Mrs Eileen Moxon, Centre for Continuing Professional Development, University of Nottingham, University Park, Nottingham NG7 2RD, United Kingdom; telephone +44 (0115) 951 3700; fax +44 (0115) 951 3722; Email Eileen.Moxon@nottingham.ac.uk.

SOFTWARE REVIEW

THE LARYNX AND CAROTID TRIANGLE — INTERACTIVE ANATOMY. VOLUME I-III

Company: Elsevier Science BV, PO Box 945, Madison Square Station, New York, NY 10159-0945. Price $415.

Hardware requirements: The CD-i version requires upgrading with a digital video extension unit (DV cartridge). The CD-ROM version requires an IBM compatible 386/33 MHz or higher processor, 4 Mb of RAM, Microsoft Windows 3.1 or higher, 3.5 Mb of hard drive space, a VGA monitor, and at least a double speed CD-ROM.

Setup: The CD-ROM version was tested on a Pentium 90 MHz system with a 4X speed CD-ROM and 8 Mb of RAM. After the initial setup, which took less than 10 minutes, the program was accessed within seconds by clicking on an icon in the Windows Program Manager.

"The Larynx and Carotid Triangle" represents the third disk in Elsevier's series of head and neck interactive anatomy disks, following "Paranasal Sinuses and Anterior Skull Base" and "Temporal Bone and Posterior Cranial Fossa." The disk contains 9,000 images of normal neck anatomy. Images of cadaver anatomy are available in the axial, sagittal, or coronal planes. In addition, noncontrasted computed tomography (CT) and magnetic resonance imaging (MRI) images are available in the same planes that correlate with the cadaver image. Histologic images of the axial section only are also presented. The CD-ROM and CD-interactive or CD-i disc contain the same information. CD-i is an uncommon format in the United States, but the publisher has included both disks in the package. CD-i may provide better color and smoother transition in the video mode. The CD-ROM version is simple to operate. After entering the program, one uses the mouse and on-screen buttons to choose the desired plane and modality (anatomy, CT, MRI, histology). The level of the section is then selected with a localization bar in the upper right corner of the window. The bar is very useful for orientation purposes. In the anatomy mode a zoom function is available; however, image clarity deteriorates with increased magnification. Structures in the anatomy mode may be identified in any plane by clicking on them with the mouse. An index of identified structures in English or Nomina Anatomica is available. Simply highlighting a name will result in an image with that structure identified with a pointer. A different level section may be accessed in ways other than using the localization bar, such as stepping one section in either direction or through the slow or fast video mode. The video mode is perhaps the most enlightening feature of the program. It can be used in any plane in the anatomy mode. With it, an image melts into the next as serial sections are displayed. Key anatomic relationships may be visualized in a dynamic fashion.

No system errors occurred during use of the program, even with multiple programs opened simultaneously. There is not a Macintosh version available. A few pauses in the program occur when one is changing section orientation while the CD-ROM accesses information; however, these are rare and usually last less than 3 seconds. The main criticism of the program is the inability to identify structures by name in the CT and MRI modes, and the loss of resolution when using the zoom function in the histology and anatomy modes.

Although the complex anatomy of the neck can only be learned through years of study and dissection, "The Larynx and Carotid Triangle — Interactive Anatomy" is a powerful educational tool that can complement the learning process. This disk will benefit all students of anatomy in understanding the structural relationships encountered in the neck.

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LETTERS TO THE EDITOR

To the Editor:

In chatting with otolaryngology colleagues, I find they seem unaware of one disturbing side effect from big-time snoring — disturbing both to patients and their bed mates, but not mentioned except on direct questioning. From my long years in gastroenterology practice, I have found that such snoring is the major cause of large-volume gastrointestinal flatus noted during the night and/or on arising! Whatever the proximal snoring site, increased resistance during exhalation facilitates esophageal air filling. Fortunately, because such flatus is just unabsorbed nitrogen, it may be curiously odor-free!

David E. Langdon, MD
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Arlington, TX 76015

To the Editor:

I am writing in regard to the article by Keithley et al (*Ann Otol Rhinol Laryngol* 1995;104:858-63). In the Discussion, the authors state, "Currently, there are no data available concerning possible changes in endolymphatic ion concentrations in humans with Meniere's disease." In my paper entitled "The Inner Ear Fluids in Man,"[1] I note that samples of endolymph collected from the vestibule just prior to labyrinthectomy for intractable Meniere's disease showed sodium (Na) and potassium (K) concentrations similar to those of normal cat endolymph. It was concluded that endolymph concentrations of Na and K were normal in Meniere's disease, while the volume was increased. These findings are consistent with the finding of Keithley et al showing normal or increased staining for ATPase in Meniere's disease.

In guinea pigs, after blockage of the endolymphatic duct, the ATPase decreases in the stria vascularis. In our study "Endolymphatic Sac Obstruction. Biochemical Studies,"[2] in which Na and K ions were analyzed after endolymphatic blockage, there was a consistent alteration in the ionic concentrations so that the Na was significantly increased while the K was decreased in the endolymph in both the vestibular and cochlear divisions. This is consistent with the findings of Keithley et al that ATPase decreased in guinea pigs after experimental hydrops was produced.

Our conclusions[2] were that the guinea pig model for Meniere's disease may not represent the human disease. Keithley et al quote the work of Sziklai et al, who found no changes in Na and K concentrations after experimental hydrops. Our work is in disagreement with that of these authors.

I am pleased that after 30 years additional evidence exists to corroborate the concept that the animal model may not represent clinical Meniere's disease.

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BOOK REVIEWS

Glue Ear in Childhood. A Prospective Study of Otitis Media With Effusion

A. Richard Maw. Hard cover, illustrated, indexed, 136 pages, 1995. Cambridge University Press, $49.95.

This book on glue ear (or by another name, otitis media with effusion) provides 15 years of research performed by Maw and his colleagues. In seven chapters he unravels the intricacies of growth of the nasopharynx and adenoids, presentation of the condition and diagnosis, treatment, and sequelae, and, most important, his conclusions. Although the sequelae of these conditions are the least known or understood aspects of them (especially in their effect on developmental and educational milestones), the most exhaustive pursuit in this book is the assessment of adenoidectomy, adenotonsillectomy, and insertion of ventilation tubes.

In regard to substance and format, the author deviates from similar volumes in that he does not dwell on bacteriology or immunology, though he refers to the subjects in an extensive and complete bibliography dating back to the late 1970s and early 1980s, when so much was written. He discusses various skeletal and soft tissue differences in the dimensions of the nasopharynx that reflect the emergence of a unique development. In summary, notwithstanding the mass of publications dealing with serous otitis media, there is no rehash of old material in this volume.

Sagacious tidbits of clinical value are present throughout the volume. It is an excellent compilation of data that will be valuable to those in training and to those who wish to review current information on selected aspects of this condition.

Dennis G. Pappas, MD
Birmingham, Alabama

Diseases of the Sinuses. A Comprehensive Textbook of Diagnosis and Treatment

M. Eric Gershwin and Gary A. Incaudo, editors. Hard cover, illustrated, indexed, 589 pages, 1996. Humana Press, Totowa, NJ, $150.

It is the goal of the authors of this text to bring to the practicing physician many of the newer concepts that can be applied in a beneficial way to patients. Indeed, the book covers a wealth of information and appears to have garnered all currently available information.

The 30 chapters are organized into four divisions: Anatomy and Physiology, Imaging, Clinical Disease, and Surgical Management. There are separate chapters for adult and pediatric sinusitis and surgery. Intricate complexities of the immune system are covered.

One of the book's strongest features is its coverage of signs and symptoms characteristic of sinusitis, analysis of the bacteria that commonly cause the disease, and illustrations that depict the disease. There is a clear hierarchy of choices of treatment. The daily dosage tables (chapter 6) of antiallergic and supplementary drugs provided are especially useful to the practitioner.

Various specialists will find sections of the book useful. The primary ones for physicians are the medically oriented chapters. Some of the photographs and tables are excellent means to convey information to nurses as well as patients. The otolaryngologist will like the coverage of surgical complications and those chapters dealing with the concepts of more unusual diseases such as cystic fibrosis and rhinocerebral mucormycosis.

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ANNALS OF OTOLOGY, RHINOLOGY & LARYNGOLOGY

— Founded in 1892 —

Volume 105
January-December 1996
Issues 1-12

Published by

ANNALS PUBLISHING COMPANY
4507 LACLEDE AVENUE
ST LOUIS, MISSOURI 63108

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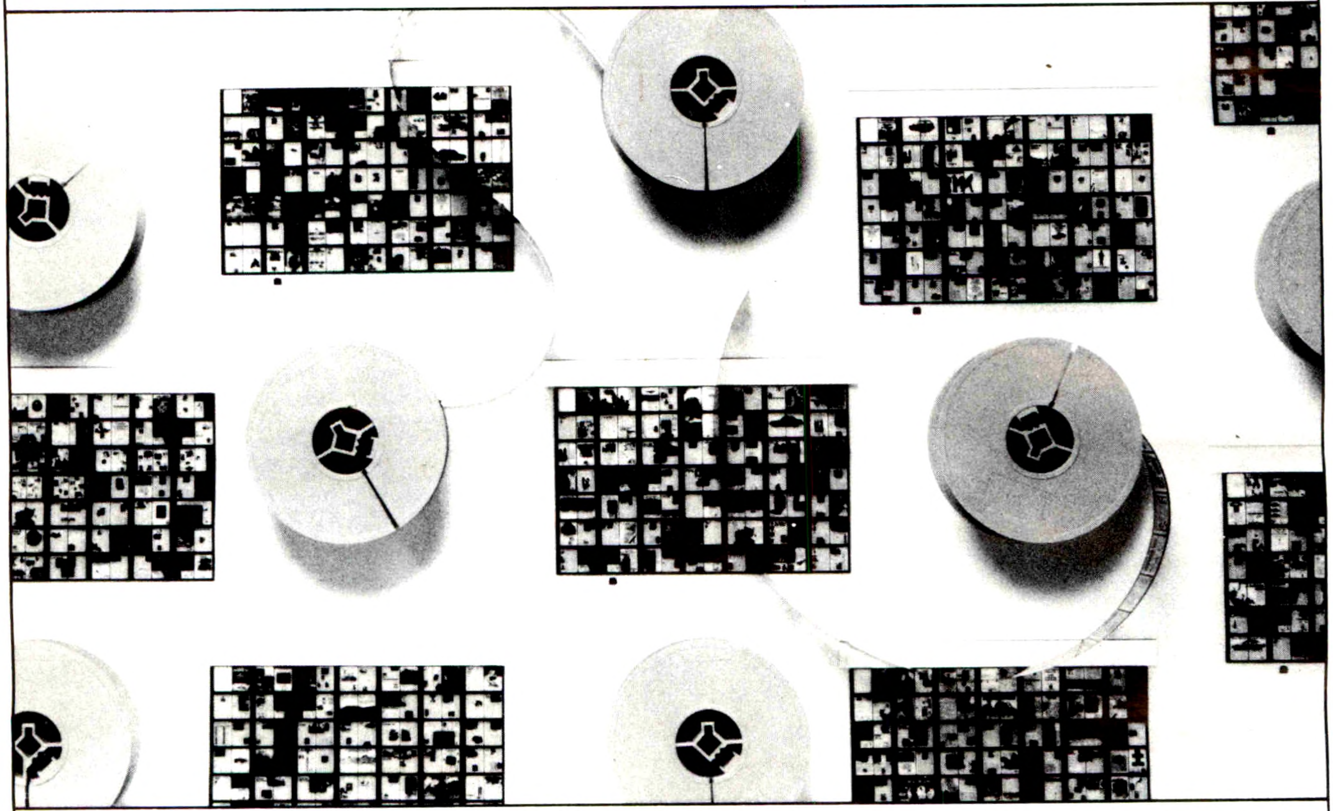

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606-010-6862A
July 1996
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